universität wien

DIPLOMARBEIT

Titel der Diplomarbeit

Verhaltensökologische Untersuchungen zur Rolle des olfaktorischen Sinns bei sekundär höhlenbrütenden Passeriformes (Sperlingsvögel)

Verfasserin

Marlies Sperandio

zur Erlangung des akademischen Grades

Magistra der Naturwissenschaften (Mag. rer. nat)

Wien, 2012

Studienkennzahl:	A 439
Studienrichtung:	Diplomstudium Biologie/Zoologie
Betreuer:	ao. Univ.-Prof. Dr. Hans-Christoph Winkler

Ich erkläre hiermit an Eides Statt, dass ich die vorliegende Arbeit selbstständig und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht. Die Arbeit wurde bisher in gleicher oder ähnlicher Form keiner anderen Prüfungsbehörde vorgelegt und auch noch nicht veröffentlicht.

Ort, Datum

Unterschrift

Inhaltsverzeichnis

What does a bird's nose know?

Observations on olfactory capacities in blue tits (*Cyanistes caeruleus*) and great tits (*Parus major)*

The scent of something new. Evidence for olfactory abilities in a passerine bird, the collared flycatcher (*Ficedula albicollis*)

Effects of pyrazine on the reproductive system of a long-distance migrant, the collared flycatcher (*Ficedula albicollis*)

Danksagung

Allen voran möchte ich mich bei ao. Univ.-Prof. Dr. Hans Winkler bedanken, der mich während der Diplomarbeit in allen Belangen betreut und tatkräftig unterstützt hat. Vielen Dank für die Geduld, besonders in statistischen Fragen, die wertvollen fachlichen Beiträge und das Ermöglichen eines selbstständigen Arbeitens.

Besonderer Dank gilt auch Prof. Harald Krenn für seinen Rat und sein Verständnis, vor allem während zeitaufwändiger Freilandtage im Frühling. Zugleich möchte ich mich recht herzlich bei Dr. Mareike Stöwe und Mag. Marlene Mann bedanken, die durch ihre hilfreiche Unterstützung während der Freilandarbeit, sowie die Zurverfügungstellung der Daten einen unerlässlichen Teil zu dieser Arbeit beigetragen haben.

Ferner möchte ich mich bei Miriam Soltész, Sebastian Feurstein und René Federspieler für das Korrekturlesen dieser Arbeit bedanken. Danke an René für die vielen hilfreichen Diskussionen und unterstützenden Worte, für seine Geduld und das große Interesse an meiner Arbeit.

Nicht zuletzt gilt ein besonders großer Dank meiner Mutter, die mich in allen Ideen und Lebenslangen unterstützt und in jeder Situation die richtigen Worte findet.

Einleitung

Bis zum heutigen Tag gilt die allgemeine Vorstellung, dass Vögel über ausgeprägte Sinnesmodalitäten im Bereich der visuellen, auditiven und propriozeptiven Wahrnehmung verfügen, die für die räumliche Orientierung im Rahmen einer fliegenden Fortbewegung unentbehrlich sind, hinsichtlich ihrer Fähigkeit zur Perzeption volatiler chemischer Substanzen aber im Laufe der Evolution eine Reduktion erfahren haben (Zelenitsky *et al*., 2011), sodass ein „Riechen" im eigentlichen Sinn, bei rezenten Arten nicht oder nur mehr begrenzt denkbar ist.

Erst in den letzten Jahrzehnten beschäftigt sich eine immer größer werdende Anzahl wissenschaftlicher Studien mit der Existenz und weiterführenden Bedeutung des Geruchssinns bei Vögeln. Mittlerweile bestätigen zahlreiche morphologische, neuroanatomische aber auch verhaltensbiologische Studien, dass viele Arten durchaus in der Lage sind, volatile chemische Substanzen in diversen biologisch relevanten Situationen wahrzunehmen (Mason & Clark, 2000). Besonders grundlegende Ergebnisse, die bis heute ihre Gültigkeit bewahren, liefert eine Studie aus den 1960er Jahren (Bang & Cobb, 1968). Die Vermessung des Bulbus olfactorius, des primären olfaktorischen Zentrums des Gehirns, von 108 Vogelarten zeigt, dass die Fähigkeit zur olfaktorischen Wahrnehmung positiv mit der relativen Größe des Bulbus korreliert und somit in manchen Arten von größerer Bedeutung ist, als in anderen (Bang & Cobb, 1968). Diese Erkenntnis hatte zur Folge, dass sich in den darauf folgenden Jahren ein großer Teil der Forschungsarbeit auf jene Arten konzentrierte, deren olfaktorisches Zentrum nachweislich in Form und Funktion dem anderer Arten überlegen ist. Wissenschaftlich besonders gut untersucht ist beispielsweise die Rolle des Geruchssinns bei der Nahrungssuche von Kiwis (Wenzel, 1968) und Truthahngeiern (Stager, 1967; Smith & Paselk, 1986), oder auch die Nutzung volatiler Substanzen bei der individuellen Erkennung und Orientierung in verschiedenen Meeresvögeln der Ordnung Procellariiformes (z.B. Nevitt, 2008), während Arten der Ordnung Passeriformes (Sperlingsvögel) lange Zeit nur wenig Beachtung in wissenschaftlichen Kreisen fanden.

Untersuchungen der letzten Jahre deuten darauf hin, dass die Bedeutung des Geruchssinns bei Sperlingsvögeln mehr als unterschätzt wurde. Umfangreiche Studien an Korsischen Blaumeisen (*Cyanistes caeruleus;* Lambrechts & Dos Santos, 2000) und Staren (*Sturnus vulgaris;* Clark & Mason, 1987) zeigen, dass diese Arten gezielt aromatische Kräuter für den Nestbau sammeln und somit nicht nur rezeptiv für verschiedene volatile Substanzen sind, sondern diese auch eindeutig unterscheiden können. Die zugrunde liegenden Mechanismen dieses Verhaltens bleiben weiterhin Spekulation, obwohl Forscher vermuten, dass die chemischen Bestandteile der Kräuter wahrscheinlich zur Abwehr von Parasiten (LaFuma, 2001), Pilzen (Adam *et al*., 1998) oder Krankheitserregern (Mennerat, 2009) und somit positiv zur Gesundheit der Nestlinge beitragen (z.B. Lambrechts & Dos Santos, 2000). Die Perzeption von Duftstoffen ist damit nicht wie bisher angenommen auf wenige Arten mit hochentwickelten olfaktorischen Gehirnzentren limitiert, sondern ist auch bei Sperlingsvögeln nachweislich eine vielseitig nutzbare Sinnesmodalität. So zeigt eine rezente Studie aus Bielefeld, dass junge Zebrafinken das eigene Nest anhand von individuellen Duftstoffen erkennen und von anderen unterscheiden können (Caspers & Krause,

2010) und der Geruchssinn wahrscheinlich auch bei der räumlichen Orientierung eine wichtige Rolle spielt. Die Passeriformes stellen innerhalb der Vögel eine einmalige Besonderheit dar, da der allgemein gültige Zusammenhang zwischen der Fähigkeit zur Perzeption von Duftstoffen und dem Ausmaß des Bulbus olfactorius nicht gegeben ist (Clark *et al*., 1993).

Ziel dieser Diplomarbeit ist es, basierend auf dem bisherigen Stand der Forschung, die Rolle des Geruchssinns bei den drei heimischen Vogelarten Blaumeise (*Cyanistes caerulues*), Kohlmeise (*Parus major*) und Halsbandschnäpper (*Ficedula albicollis*) im Rahmen von verhaltensbiologischen Freilandversuchen in Teilaspekten zu untersuchen und somit einen Beitrag zur wissenschaftlichen Forschung in diesem Bereich zu leisten. Alle Freilandversuche fanden in den Monaten März bis Juni 2011 in den Arealen Kolbeterberg, Buchberg und Wilhelminenberg des Wienerwaldes statt. In diesen Untersuchungsgebieten sind eine große Anzahl Nistkästen für gemeinsame Studienzwecke des KLIVV (Konrad Lorenz Institut für vergleichende Verhaltensforschung), des FIWI (Forschungsinstitut für Wildtierkunde und Ökologie) und der Veterinärmedizinischen Universität angebracht. Die weitläufigen Buchen- und Hainbuchenwälder im nordöstlichen Teil des Wienerwaldes stellen für viele heimische Brutvögel einen idealen Lebensraum mit konstanten Habitatbedingungen dar, nicht zuletzt da der Wienerwald auf gemeinsame Initiative der Bundesländer Niederösterreich und Wien seit 2005 als Biosphärenpark von der UNESCO anerkannt wird. Nach jahrzehntelanger forstwirtschaftlicher Nutzung ist der Wald heute trotz der Schutzmaßnahmen arm an Totholz, Altholzbeständen und somit natürlichen Brutmöglichkeiten. Durch die Anbringung von Nistkästen können geeignete Brutplätze für höhlenbrütende Arten wie Kohlmeisen (*Parus major*), Blaumeisen (*Cyanistes caeruleus*), Halsbandschnäpper (*Ficedula albicollis*) und Spechte geschaffen werden, wobei die drei erstgenannten Arten, wie eingangs erwähnt, Hauptsubjekt dieser Studie waren. Die Nistkästen des Untersuchungsgebiets werden alljährlich von lokalen Populationen sekundärer Höhlenbrüter als Nistplatz akzeptiert, was langfristige Bestandsüberwachungen, Untersuchungen zum Einfluss von Umweltfaktoren auf Gelege und Nestlinge und auch verhaltensbiologische Studien ermöglicht.

Im Rahmen dieser Arbeit wurden mehrere unabhängige Untersuchungen zur Rolle des Geruchssinns bei sekundär höhlenbrütenden Spezies durchgeführt. Bedingt durch die Diversität der untersuchten Arten bezüglich ihrer Ökologie, aber auch wichtiger life-history Parameter findet hinsichtlich der formalen Präsentation eine Aufteilung in drei konzeptionell homogene Teilgebiete statt, die jeweils als inhaltlich geschlossenes Manuskript abgehandelt werden. Eine Veröffentlichung der Ergebnisse ist zumindest teilweise geplant, weshalb die Arbeit in Englischer Sprache verfasst ist.

Präzisierte Fragestellungen, Methoden und alle weiterführenden Ergebnisse werden im Hauptteil dieser Arbeit ausführlich behandelt. Um einen thematischen Abriss der einzelnen Bereiche zu ermöglichen, sind im Folgenden die Inhalte zusammenfassend dargestellt.

1. What does a bird's nose know? Observations on olfactory capacities in blue tits (Cyanistes caeruleus) and great tits (Parus major)

Die Fähigkeit zur Wahrnehmung von Duftstoffen ist innerhalb der Passeriformes nur bei wenigen Arten, wie z.B. Blaumeisen (*Cyanistes caeruleus;* Lambrechts & Dos Santos, 2000), Staren (*Sturnus vulgaris;* Clark & Mason, 1987) und Zebrafinken (*Taeniopygia guttat*a; Casper & Krause, 2010) wissenschaftlich bestätigt. Die Inhalte dieser Studie fokussieren auf den Nachweis eines olfaktorischen Sinns bei einer bisher nicht untersuchten Art aus der Familie der Paridae, der Kohlmeise (*Parus majo*r), wobei Verhaltensbeobachtungen nach experimenteller Änderung des Nestgeruchs an 21 Nestern der lokalen Kohlmeisenpopulation Aufschluss über eine mögliche Rezeptivität adulter Vögel für volatile Substanzen geben sollen. Für sekundäre Höhlenbrüter eröffnet die Verfügbarkeit einer zusätzlichen Sinnesmodalität eine Vielzahl neuer und bisher unbekannter Möglichkeiten in grundlegenden Interaktionen mit ihrer Umwelt.
Gleichzeitig soll anhand einer lokalen Population von Blaumeisen, deren Rezeptivität für volatile Substanzen bereits mehrfach nachgewiesen ist, eine mögliche Relevanz des Geruchssinns einerseits im Kontext der Prädatorenerkennung und andererseits hinsichtlich eines möglichen Langzeiteffekts auf elterliches Investment aufgezeigt werden. Diese Untersuchungen sollen vor allem einen wichtigen Beitrag zur methodischen Grundlagenforschung leisten und Aufschluss über die olfaktorische Rezeptivität einer bisher nicht untersuchten Art geben.

2. The scent of something new. Evidence for olfactory abilities in a passerine bird, the collared flycatcher (Ficedula albicollis)

Halsbandschnäpper (*Ficedula albicollis*), eine Art der Familie der Muscicapidae, gelten in wissenschaftlichen Kreisen bis heute als anosmisch. Als sekundäre Höhlenbrüter nützen sie europäische Laubwälder als geeignetes Brutgebiet, ziehen im Herbst zum Überwintern in das zentrale oder südliche Afrika und unterscheiden sich damit hinsichtlich ihrer Ökologie und Brutbiologie grundlegend von vielen heimischen Standvögeln.
Unter Verwendung eines methodisch identen Ansatzes aus der vorangegangenen Studie, liegt der Fokus dieser Teilarbeit auf der Untersuchung einer potentiellen Rezeptivität für volatile Substanzen bei Halsbandschnäppern. Die Ergebnisse der Studie werden vor dem Hintergrund der ökologischen Relevanz des Geruchssinns im Leben eines Langstreckenziehers präsentiert und vergleichend diskutiert.

3. Effects of pyrazine on the reproductive system of a long-distance migrant, the collared flycatcher (*Ficedula albicollis*)

Aufbauend auf den bisherigen Ergebnissen dieser Arbeit untersucht diese Freilandstudie die Auswirkungen eines externen Duftstoffs auf physiologische Vorgänge innerhalb des Organismus. Forschungsarbeiten beweisen, dass der persistente Geruch von Pyrazin ($C_4H_4N_2$) wichtige Funktionen innerhalb des Reproduktionsapparates von Haushühnern (*Gallus gallus*) maßgeblich beeinflussen kann und zu einem signifikanten Anstieg des mittleren Ei- und Nestlingsgewichts führt (Barnea & Rothschild, 2002).

Im Rahmen dieser Untersuchung an 26 Nestern der lokalen Population von Halsbandschnäppern werden Daten erhoben, die grundlegende Schlüsse über die mögliche Auswirkung eines externen Odors (Pyrazin) auf die Gelegegröße, das Eigewicht und Nestlingsgewicht von Halsbandschnäppern zulassen sollen. Die Ergebnisse der Studie, sowie basale Mechanismen und eine mögliche Relevanz des ubiquitären chemischen Stoffes Pyrazin im Leben eines Langstreckenziehers werden kritisch dargestellt diskutiert.

What does a bird's nose know? Observations on olfactory capacities in blue tits (*Cyanistes caeruleus*) and great tits (*Parus major*)

Abstract

Among most terrestrial vertebrates, olfaction is a well-developed sense that mediates environmental interactions in a variety of biologically relevant contexts. Due to historical misconceptions olfactory prowess in birds still remains sparsely investigated, especially within species of the order Passeriformes who are noted to posses the smallest olfactory bulbs in the avian kingdom. In the breeding season of 2011 a set of experiments was conducted to examine olfactory abilities in three passerine species. The first part of the study aimed to test new experimental approaches for in field studies under semi-natural conditions, by evaluating potential odor dependent preferences in selection of nesting material in blue tits (*Cyanistes caeruleus*) and monitoring long-term effects of nest odor manipulation in the same species.

Major work focused on the existence of olfactory abilities in another passerine, the great tit (*Parus major*), by manipulating nest odor composition and monitoring short-term behavioral responses. A total of 21 nests of a local free-ranging population of blue tits were randomly assigned to two experimental groups. One group was exposed to the experimental odor of *Lavandula officinalis*, while the other acted as a control and was treated with water only. A potential effect of odor treatment was measured as reflected in variations of latency before entering the nestbox or time spent inside the nest. Results bring evidence that great tits are receptive to volatile compounds added artificially to the nest. Results and possible ecological or evolutionary mechanisms for the existence of an olfactory sense in a passerine bird are presented and discussed critically.

1. Introduction

Olfaction or the sense of smell is generally described as the ability to perceive information from the environment by sampling the surrounding for volatile chemical substances (Nef, 1998). In being a highly versatile chemical sense, olfaction is of primary importance in many vertebrates and mediates interactions with the environment in multiple biologically relevant contexts, e.g. foraging, individual recognition, communication, mating, orientation, territorial marking or predator recognition and avoidance (Ache & Young, 2005). Even more surprising that the historical misconception that birds have little or no sense of smell, relying rather on vision and acoustics as major sensory modalities (Roper, 1999), is still a commonly held belief.

However, extensive neurophysiological and anatomical analyses of avian brains point to the fact that the avian olfactory sense is almost on the same level with that in mammals, as stated by Mason & Clark (2000). Comprehensive studies show that the avian olfactory system comprises all fundamental features essential for remote chemoreception and displays considerable similarities with the typical vertebrate structure (Roper, 1999). In general, inhaled air passes through paired external nares into a system of nasal chambers, where volatile chemical stimuli contact with olfactory sensory neurons in the olfactory epithelium. Binding of distinct odorant molecules to olfactory receptors of the epithelium triggers the generation of action potentials, which are transmitted to the olfactory bulb via paired olfactory nerves (e.g. Lledo *et al*., 2005). Electrophysiological studies of Rieke & Wenzel 1978 (as reviewed by Balthazar & Taziaux, 2009) on pigeon brains demonstrated that the olfactory bulb in turn projects to various telencephalic loci and further on to different behavior-relevant brain areas, that are potentially able to process the received information. Accurate functioning of this mechanism is experimentally assured, since different studies under laboratory as well as natural conditions supply detailed evidence that the presence of an olfactory stimulus elicits behavioral responses in a variety of situations.

In 1968 Bang & Cobb measured the relative olfactory bulb size in 108 different species of birds, presuming that an evolutionary enlargement of a part of the brain implies an upgrade in function (Bang & Cobb, 1968; Bang, 1971). Measurements showed that ratios of olfactory bulbs to cerebral hemispheres varied substantially among species, since the olfactory bulb took up more than 30% in the brains of different tube-nosed seabirds and only 3% in a passerine (Bang & Cobb, 1968). Following the conclusion size entails function, the olfactory sense seemed to be of primary importance in species with great ratios over 28% (Buitron & Nuechterlein, 1985) and is, based on little relative olfactory bulb ratios, relatively unimportant in other groups like the Passeriformes (Bang & Cobb, 1968). This interpretation is consistent with findings of various field experiments, which show that species known to possess large olfactory bulbs, like Procellariiformes (e.g. Bonadonna *et al*., 2003) or kiwis (Wenzel, 1968) use olfaction in distinct behavioral contexts. Therefore, the size of the olfactory bulb can be seen as anatomical 'benchmark' for the extent of olfactory capacities in bird species, except in passerines, where no correlation between size of olfactory tissue and function is to be found (Clark *et al.*, 1993). In this context, one has to consider the findings of Steiger *et al.* (2008), who discovered a significant causal relationship between the total number of genes in the avian genome encoding for olfactory receptors (OR genes) and the relative size of the olfactory bulb in different bird species. In 2006 Niimura & Nei suggested that neither the total number of OR genes, nor the amount of functional OR genes in the genome of mammals may solely be seen as predictors of olfactory acuity. However, OR genes are involved in the most basal processes of olfaction and are nevertheless the most reliable and important factors for estimations on olfactory capacities (Niimura & Nei, 2006). Steiger *et al.* (2008) who investigated OR genes in nine bird species found that the proportion of potentially functional OR genes was significantly higher than originally expected, indicating a well-developed and accurate sense of smell in birds.

Nowadays, a steadily increasing amount of research is done to examine the olfactory sense and its biological relevance in birds, bringing evidence for high olfactory sensitivity and well-developed discrimination capabilities in distinct species of the avian kingdom. Avian olfactory capacities are particularly well studied (e.g. for reviews, see Roper, 1999; Balthazart & Taziaux, 2009) in the context of orientation in homing pigeons (Papi *et al.*, 1971), nest maintenance in Corsican blue tits (e.g. Mennerat, 2008) and starlings (Clarke & Mason, 1987), foraging in kakapos (Hagelin, 2004), kiwis (Wenzel, 1968), new world vultures (Stager, 1967; Smith, 1986) and procellariiform seabirds (e.g. Nevitt, 2008). The latter possess highly sophisticated sensory abilities that offer unequaled opportunities to their owner in a variety of environmental interactions, e.g. foraging at sea, relocation of nesting burrows or individual recognition (Bonadonna *et al.*, 2003; Bonadonna & Nevitt, 2011; Nevitt, 2008).

Although the use of olfaction is no longer questioned its relevance in one order of the avian kingdom, the Passeriformes, which includes more than half of all bird species known (Martin, 1986), remains poorly investigated. Literature pertaining to the olfactory sense in passerines is scarce but convincing, pointing to the fact that odorous stimuli play a major role in nest building and maintenance of different species. In 1987 Clark & Mason found that European starlings (*Sturnus vulgaris*) are able to use volatile cues to discriminate between fresh plant material, since starlings, like few other passerines, incorporate fresh plant fragments preferably rich in volatile compounds (Gwinner & Berger, 2006) into their nests during nesting period (Wimberger, 1984). On Corsica, females of cavity-breeding blue tits (*Cyanistes caeruleus*) daily add fresh aromatic herbaceous plants to their nests (Lambrechts & Dos Santos, 2000) and are able to discriminate between different plant volatile compounds (Petit *et a*l., 2002). Five distinct plant species have been identified to be most frequently found in nests of blue tits: *Achillea ligustica, Lavandula stoechas, Mentha suaveolens, Pulicaria odora* and *Helichrysum italicum* (Petit *et al.*, 2002). Recent findings (Mennerat, 2008) under semi-natural conditions suggest that blue tits are capable of detecting a change in the aromatic composition from outside the nest cavity. After experimental manipulation by adding aromatic plant fragments to the nest, birds of both sexes showed a significant increase in latency before entering the cavity. Mechanisms underlying this behavior remain unknown, although different studies indicate that chemical compounds of aromatic plants repel or kill different nest parasites (Mennerat *et al*., 2009) or decrease the amount of bacteria inside the nest (LaFuma *et al*., 2001).

In the breeding season of 2011 a set of experiments under semi-natural conditions was conducted to examine the role of olfaction in three species of passerines, only one of them, the blue tits, known to incorporate fresh plant material into their nests. Since the ability to perceive volatile substances has already been demonstrated in blue tits, a first trial contributing to fundamental research aimed to test a new methodical approach under semi-natural conditions and investigated the potential relevance of olfaction in one of the most important determinants of bird survival, predator avoidance. Hence, an experimental setup was designed to determine a possible odor dependent choice of nesting material in blue tits. Since snakes, like the Aesculapian snake (*Elaphe longissima*), presumably account for a majority of losses in clutches and nestlings of cavity-breeding birds, a potential snake-repellent odor seemed appropriate in this context. The experimentally applied aromatic herbs *Achillea millefolium* and *Lavandula officinalis* are known to be common elements used for nest construction in Corsican blue tits. The relevance of lavender in this context is bivalent, since distinct compounds of this herbaceous species are proven to be effective repellents for brown tree snakes (Clark & Shivik, 2002). It is expected that blue tits supposably prefer aromatic nesting material over the odorless control group, whereas specific selection eventually allows conclusion on the biological relevance of odors in nest maintenance. Results of this experiment contribute to fundamental research and introduce a new methodological approach for investigations on free-living populations.

In a second experiment long-time effects of aromatic odor treatment of the nest on parental investment in blue tits were investigated. Different studies examining use of aromatic plants in nest maintenance suggest, that rising complexity of odor bouquet contributes to higher probability of nestling survival, since a variety of potential parasites (LaFuma, 2001) and bacteria (Mennerat *et al*., 2009) are killed by distinct chemical plant compounds. If incorporation of aromatic nesting material by female birds somehow contributes to nestling fitness by either decreasing parasite load or predation risk, one could speculate that male birds are encouraged to invest more in offspring fitness and enhance feeding effort. By artificially manipulating nest odor, this study aimed to examine whether (1) long-time effects of aromatic manipulation on parental behavior are detectable, and (2) whether parental investment is correlated to complexity of nest odor composition. Over three consecutive days of experimental observation, behavioral effects are expected to reflect in variations of feeding intervals, e.g. time elapsed between approaches to the nestbox for a feeding purpose. Data is supposed to offer valuable clues to the presumed correlation between nest odor complexity and long-time effects on parental investment.

Main task of this research project was focused on olfactory abilities in another member of the passerine family, the blue tit (*Parus major*). Preliminary results earlier this spring bring evidence that Collared flycatchers (*Ficedula albicollis*), although naturally not using green herbaceous nest material, are sensitive to volatile compounds added artificially to the nestbox. Encouraged by these findings and the results of Mennerat (2008), another experiment was designed to investigate the role of olfaction in a free-ranging population of great tits by manipulating nest odor composition and monitoring short-term behavioral responses. Possible behavioral effects of odor treatment are expected to reflect in a variation of latency before entering the nest cavity or variations in times spent inside the nestbox. It is estimated that after odor manipulation of the nest adult great tits of both sexes, if receptive to odors, will engage in cautious behavior either by elongating latency before entering or time spent in the nestbox. Results and feasible mechanisms for these effects will be critically discussed with regard to biological relevance of the olfactory sense in the life of a residential passerine bird.

2. Materials and Methods

Experiments were conducted in the breeding season of 2011 in the north-eastern part of the Wienerwald in Vienna (Austria), where nestboxes are accepted for breeding by blue tits (*Cyanistes caeruleus*) and great tits (*Parus major*). These birds of the family Paridae (Passeriformes) are common residents in the temperate climate of central Europe. Both species are secondary cavity nesters, preferring mixed deciduous woodlands for breeding. Mating, nest construction, egg deposition and breeding initiates by end of March and continues until mid June. Clutch sizes range from 5 to 15 in blue tits, and 5 to 10 in great tits. From the onset of nest building the nestboxes were monitored regularly to examine the progress of nest construction, the onset of egg laying, hatching date and number and constitution of the nestlings until fledging.

2.1 Odor dependent choice of nesting material in blue tits

Trials for odor dependent selection of nesting material were carried out on a total sample of 21 nests in the areas of Kolbeterberg, Buchberg and Wilhelminenberg on the local free-ranging population of blue tits. Prior to the experiment, major amounts of dog's hair were collected and washed to reduce the characteristic inherent smell. Dog hair was proposed suitable experimental material since it is well known that blue tits incorporate hair and undercoat in their nests to stuff the inner of the nest scrape. Dry hair was portioned and weighed and filled into fine nets to form balls. A composite of three balls was fixed to a branch in approximately 2-3 meters distance to each nest. The aromatic treatment consisted of adding an equal amount (3 drops) of different essential oils (Primavera Life GmbH) to the balls, at which each treatment group consisted of a lavender (*Lavandula officinalis*), a milfoil (*Achillea millefolium*) and a water treated control ball. Application of essential oils seemed appropriate, since testing conditions could be standardized and odor intensity is known to be long-term persistent. The experimental composite was installed at the onset of building the nest scrape and was removed 48h later. Initial filling amounts of each ball were compared with amount of dog's hair after the experiment to assess withdrawal quantities. All nestboxes in proximity to the experimental setups were examined visually and olfactory for contents of dog's hair immediately after completion of trials.
The aromatic plant species lavender and milfoil are known to play a major role in nesting behavior

of Corsican blue tits (e.g., Petit *et al*., 2002; Mennerat, 2008). Gas chromatography (Lambrechts & Hossaert-McKey, 2006) demonstrates distinct variances of volatile compound composition in these plants, suggesting the possibility for blue tits to discriminate between the species. Furthermore, lavender oil was identified (Clark & Shivik, 2002) to be an effective repellent for brown treesnakes (*Boiga irregularis*).
Differences in net weight were log transformed prior to analysis to meet assumptions of normality. A general linear model with treatment and location as fixed factors and weight difference as dependent variable was performed. All statistical analyses were done using SPSS (version 16.0) software.

2.2 Long-time effects of nest odor manipulation on parental investment in blue tits

For assessment of long-time effects of nest odor manipulation on parental investment, a total number of 22 nests of a local population of blue tits was assigned for the experiment. Behavioral response was measured as reflected in a change of feeding intervals of parents on two consecutive days following an experimental treatment.
Nests were randomly divided into three experimental groups: group one (n=8) was exposed to the odor of thyme (*Thymus vulgaris*) only, group two (n=7) to a composite of thyme (*Thymus vulgaris*), mint (*Mentha viridis*) and lavender (*Lavandula officinalis*), while group three (n=7) acted as a control and was treated with water only. Experimental treatment consisted of adding 1 drop of essential oil (Primavera Life GmbH) with distinctive odors as main components directly to the nest.
Behavioral observation consisted of recording the feeding intervals (s) of adult birds, i.e. time elapsed between entrances to the nestbox for a feeding purpose, for 30 minutes. Trials initiated when nestlings reached the age of day 6-7 post hatching. Preliminarily to the experimental treatment, feeding intervals of adult birds were examined under natural conditions, followed by either odor or water application. Observations were repeated on two consecutive days following the experimental treatment in a precise 24h interval.
All observations were done at approximately 20-30 m distance to the nestbox with the help of a spotting scope and making use of natural shelters. Nestboxes were not opened until both parents had left for a foraging sortie and disappeared completely from the observers sight. Under given experimental conditions, in-field discrimination of sexes was vaguely possible, since plumage coloration of male and female blue tits is fairly homogenous. For achievement of reliable results, feeding intervals therefore refer to the time elapsed between approach of an adult bird to the nestbox, disregarding sexes.
The aromatic plants of thyme, mint and lavender belong to the main components frequently used by Corsican blue tits for a nesting purpose (Petit *et al*., 2002). The use of essential oils facilitates standardized testing conditions and guarantees reproducibility of experiments.
Data for timespans between feeding approaches were log transformed prior to statistical analysis. Since the bird's behavior on each day of trial is not independent from each other, a paired t-test

was conducted for comparison of feeding intervals on consecutive days and between treatment groups. Mixed effect models (ANOVA) were performed with treatment as fixed factor to show effects of experimental treatment on feeding intervals. Statistical analyses were done using SPSS (version 16.0) software.

2.3 Olfactory capacities in great tits

Trials were carried out in the area of Kolbeterberg on a total sample of 17 nests (days 10-12 post hatching), assigned randomly to two experimental groups: one group was exposed to an experimental odor (n=9) while the other acted as a control (n=8). The aromatic treatment consisted of 1 drop of essential oil (Primavera Life GmbH) with lavender (*Lavandula officinalis*) as main component, the control treatment of 1 drop of water, added to 2g of moss. Nests of the treatment group were provided with fragranted moss, nests of the control group with moisture moss only, which was hidden in the rear part of the nest to avoid premature removal by adult birds or direct contact of nestlings with the odor. Application of essential oils seemed appropriate, since dosage can be standardized and testing conditions are replicable. Therefore the amount of odorous substances added was constant in each nest of the treatment group. All experimentally added materials were removed directly after the experiment.

Lavender is without known biological significance to the tested species, but is verified to be incorporated by Corsican blue tits (*Cyanistes caeruleus*) into their nests (Petit *et al.*, 2002), demonstrating a sensitivity of olfactory receptors to the component in this species. The obvious relevance of lavender as olfactory stimulus in a related biological context and lack of any discernible irritating components justifies the use in this experiment.

All observations were performed at approximately 20-30 m from the nestboxes by using a spotting scope and making use of natural shelters, e.g. trees, trunks or rocks. To keep disturbance to a minimum, nestboxes were not opened for experimental treatment until both parents had left the box and disappeared completely from the observer's sight. Recognition between sexes during the experiment was sparsely alleviated by the fact of sexualdimorphism in plumage coloration. Females are slightly duller than males and the black mid-line that extends on the front from bib to vent is narrower and often discontinuous in female birds. Although this distinctive feature was clearly visible when a bird exited the nestbox, inaccurate discrimination of sexes biasing final results can not be entirely excluded.

Behavioral observation initiated immediately after application of the treatment and consisted of recording (1) the number of unsuccessful visits of each parent before entering the nestbox, (2) latency (LT) of male and female before entering the nestbox and (3) the time spent in the nestbox (in-nest-time, INT) by each parent. Preliminary to the actual experiment, all nests were monitored without experimental influence to determine the natural behavior of birds. All variables were therefore measured under natural conditions before and experimental conditions after treatment. An unsuccessful visit occurred, when a bird approached the nestbox and left immediately without

entering the cavity. Latency or hesitation time was defined as the time in which the bird was in direct physical contact with the nestbox without entering. Recording of INT and LT stopped after 10 successful visits, i.e. entrances to the nestbox for a feeding purpose. Monitoring was performed by one single observer, times (in seconds) were recorded with a stopwatch.
Prior to statistical analysis latency and in-nest-times were log plus one transformed to approximate a normal distribution. An analysis of covariance (ANCOVA) was performed with LT and INT as dependent variable and visit order (visits 1-10) as covariat, to correct results from the influence of habituation. Treatment, sex and time of assessment (before/after treatment) were included as fixed factors. Results show possible differences in LT and INT before and after treatment, between sexes and treatment groups with respect to a distinct habituation. For achievement of improved comparability of LT and INT over successive visits linear regression models were applied and Spearman's rank correlation coefficient was employed as method for comparative analysis. All statistical analyses were done using SPSS (version 16.0) software.

3. Results

3.1 Odor dependent choice of nesting material in blue tits

Results show (Fig. 1) that withdrawal quantities of dog's hair did not differ significantly between the experimental groups (F=0.415, p=0.663). Hence, birds did not show an odor specific preference in the context of acquisition of nesting material.

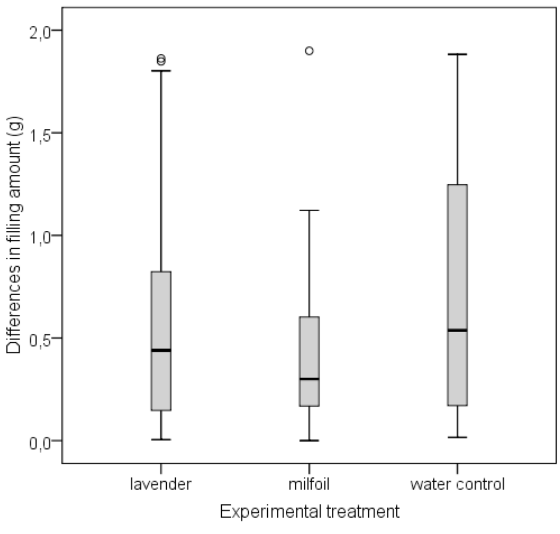

Fig. 1 Withdrawal quantities of nesting material (g) in the three experimental groups treated with lavender, milfoil or water only in a total number of 21 nests.

Withdrawal quantities vary slightly between the experimental groups but effects failed to be statistically significant. A slight trend in increase of withdrawal amounts in the water control group can be seen in Figure 1 and Table 1. Mean amount of dog's hair taken for nesting purpose was highest in the water control group, though not significantly.

Tab. 1 Descriptive data analysis of withdrawal quantities in lavender-treated and milfoil-treated nesting material as well as the water control group. Mean amount of dog's hair taken for nesting purpose was highest in the water control group.

	Mean (s)	Median (s)	Variance (s)	Std.dev. (s)
lavender	0.63	0.44	0.42	0.64
milfoil	0.45	0.30	0.23	0.48
water control	0.76	0.54	0.44	0.66

Further investigation of nestboxes situated in the study areas revealed that the experimentally offered nesting material was incorporated into the nest scrape in a total of 22 nests of blue tits. Olfactory discrimination showed that lavender treated hair was only used in 2 nests, milfoil treated hair in 6 nests, while a mix of both odors could be identified in 10 nests. Hair of the water control group was incorporated into 6 nests. Interestingly, adult blue tits tended to exaggerate hair acquisition since many nestboxes were stuffed completely with experimental dog's hair. Deteriorating for final results, experimental nesting material could also be located in 10 nests of great tits, implying that the experimentally offered nesting material was also used by other species than the blue tit.

3.2 Long-time effects of nest odor manipulation on parental investment in blue tits

A direct comparison of feeding intervals in the water control group on three days of trial period showed, that time elapsed between feeding approaches remained constant on day two ($t=-0.944$, $p=0.381$) and day three ($t=0.788$, $p=0.461$) in comparison to feeding intervals of the preceding day. Equal results are given in the experimental group treated with a composition of three odors, since feeding intervals did not differ on day two ($t=0.543$, $p=0.607$) or day three ($t=0.213$, $p=0.838$). Hence, feeding intervals remained constant over the testing period in the water treated control group as well as the mixture group. In nests treated with mint only, time elapsed between each feeding approach was significantly longer on the second day in comparison to feeding intervals under natural conditions ($t=0.3450$, $p=0.011$), and remain constant on day 3 of trials ($t=1.104$, $p=0,306$). Results are given in Figure 2.

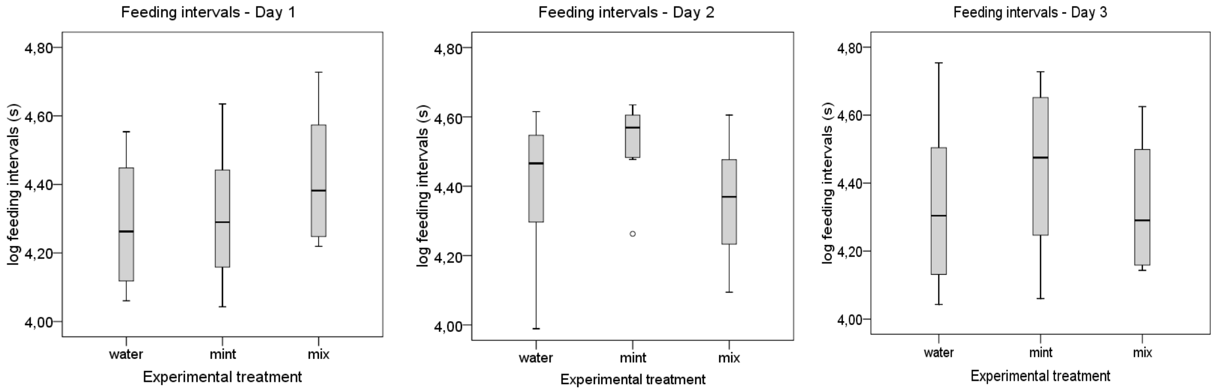

Fig. 2 Effects of experimental treatment on time elapsed between feeding approaches of adult blue tits on day two and three of trials, in comparison to feeding intervals under natural conditions (day 1).

Comparison of feeding intervals on individual days of trial shows, that time periods between approaches of adult birds for a feeding purpose are constant under natural conditions in all nests ($F=0.992$, $p=0.389$), and do not vary within experimental groups on day two ($F=1.876$; $p=0.180$) and three ($F=0.757$, $p=0.483$).

However, table 2 shows that mean feeding intervals in the water control group as well as the mint-treated group tend to be considerably longer – hence not significantly – on the second day, indicating that adult blue tits less frequently return to the nestbox for a feeding purpose. On day three of trials mean time elapsed between feeding approaches decreases slightly in all three experimental groups.

Tab. 2 Descriptive data analysis of feeding intervals in three experimental groups over the testing period (day 1-3). Std. dev.... Standard deviation.

		Mean (s)	Median (s)	Variance (s)	Std. dev. (s)
Day 1	water-treated	74,0	71,0	216,0	14,7
	mint-treated	75,5	73,0	226,0	15,0
	mix-treated	85,0	80,0	315,7	17,8
Day 2	water-treated	82,7	87,0	304,9	17,5
	mint-treated	93,0	96,5	108,6	10,4
	mix-treated	79,0	79,0	202,3	14,2
Day 3	water-treated	75,0	74,0	782,0	27,9
	mint-treated	87,1	88,0	421,0	20,5
	mix-treated	78,0	73,0	256,3	16,0

3.3 Olfactory capacities in great tits

Latency before entering the nestboxes was significantly higher in birds of both sexes after the aromatic treatment than after water treatment (male: F=14.498, p<0.0001; female: F=17.483, p<0.0001). Hence, birds of both sexes hesitated significantly longer to enter the cavity after nest odor manipulation (Tab. 3). This distinct effect was highest in the first few visits (Fig. 3) after aromatic treatment and decreased significantly over successive approaches in male (F=35.898, p<0.0001) and female (F=17.483, p<0.0001), reflected also in altered Spearman's rank correlation coefficients (Tab.6).

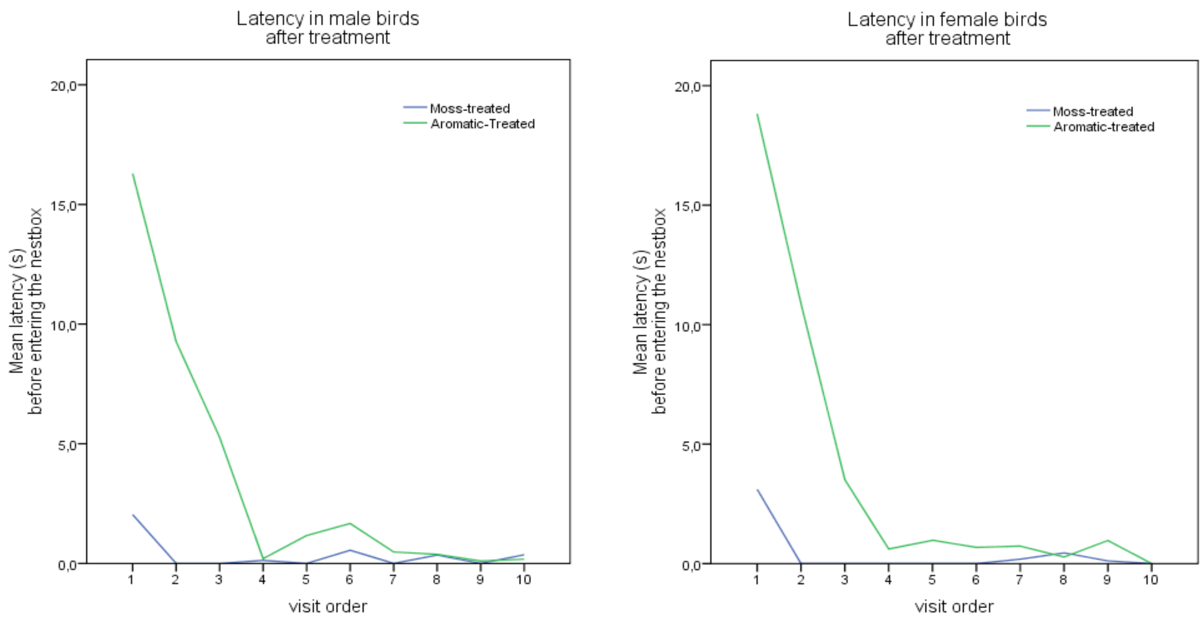

Fig. 3 Mean latency (s) before entering the nestbox in adult birds over 10 successive visits in moss-treated control nests and aromatic-treated nests. Hesitation times are significantly longer after aromatic treatment in both sexes.

Tab. 3 Effects of visit order and treatment, before and after experimental treatment on latency and times spend in the nestbox in male and female birds, as tested by analysis of covariance (ANCOVA).
d.f. … degrees of freedom

			Latency before entering				In-nest-times			
			Male		Female		Male		Female	
		d.f.	F	p	F	p	F	p	F	p
Before treatment	visit order	1	1.053	0.306	0.317	0.574	0.016	0.898	7.788	0.006
	treatment	1	3.257	0.073	3.683	0.057	5.048	0.126	10.375	0.002
After treatment	visit order	1	35.898	<0.0001	35.846	<0.0001	17.043	<0.0001	21.193	<0.0001
	treatment	1	14.498	<0.0001	17.483	<0.0001	2.604	0.109	0.007	0.934

Unsuccessful approaches of birds to the nestboxes were exclusively observed after odor treatment (n=9) with a mean number of 1.7 unsuccessful approaches in male and 2.0 in female. The behavior of approach and instant departure without entering the nest cavity only occurred in the time period immediately following treatment and reached a maximum of 5 unsuccessful visits in male and 7 in female. Natural approach behavior, e.g. approach followed by entry of the cavity for feeding purpose, was restored and maintained when the bird accomplished a first successive entry to the nestbox. Unsuccessful approaches could neither be observed under natural conditions preceding the experiment nor after water treatment in the control group.

Preliminary to treatment application latency was equal in treatment groups (Tab. 3) and sexes (Tab. 4), indicating that approach behavior in male and female resembles under natural conditions and remains consistent within birds assigned for the experiment. Behavioral response to nest odor manipulation as reflected in hesitation time did not differ between the sexes (F=0.213, p=0.645) but decreased significantly over successive visits (F=69.409, p<0.0001).

Tab. 4 Effects of visit order and sex, before and after experimental treatment on latency and times spend in the nestbox in moss-treated and aromatic-treated nests, as tested by analysis of covariance (ANCOVA).
d.f. … degrees of freedom

			Latency before entering				In-nest-times			
			Moss-treated		Aromatic-treated		Moss-treated		Aromatic-treated	
		d.f.	F	p	F	p	F	p	F	p
Before treatment	visit order	1	2.467	0.118	0.129	0.720	4.808	0.130	0.879	0.352
	sex	1	0.148	0.701	0.013	0.910	38.264	<0.0001	27.405	<0.0001
After treatment	visit order	1	10.502	0.001	69.409	<0.0001	5.125	0.025	42.846	<0.0001
	sex	1	0.158	0.692	0.213	0.645	27.832	<0.0001	57.737	<0.0001

Latency was significantly longer (Tab. 5) after odor manipulation than under natural conditions preliminarily to the experimental treatment (male F=9.129, p<0.003; female F=11.300, p<0.001). However, birds assigned to the moss control group did not hesitate longer before entering the nest cavity after the experimental treatment although behavioral modifications possibly due to observer effects immediately after moss-treatment, reflected in longer latency in the first visits (male F=8.626, p=0.004; female F=4.583, p=0.034), were detectable.

Tab. 5 Effects of visit order and time of assessment (before/after treatment), in moss-treated and aromatic-treated nests on latency and times spend in the nest-box in male and female birds, as tested by analysis of covariance (ANCOVA).
d.f. … degrees of freedom

			Latency before entering				In-nest-times			
			Male		Female		Male		Female	
		d.f.	F	p	F	p	F	p	F	p
Moss-treated	visit order	1	8.626	0.004	4.583	0.034	1.041	0.309	10.278	0.002
	before/after	1	0.469	0.494	1.792	0.183	0.841	0.361	0.363	0.548
Aromatic-treated	visit order	1	24.871	<0.0001	17.429	<0.0001	8.705	0.004	18.088	<0.0001
	before/after	1	9.129	0.003	11.300	0.001	3.408	0.067	13.186	<0.0001

Spearman's rank correlation test (Tab. 6) shows a highly significant negative correlation between latency and visit order after treatment in sexes and treatment groups, supporting the assumption that hesitation times were longest in the first few visits and decrease over successive approaches. In the control group correlation between latency and visit order after aromatic odor manipulation of the nest is not significant.

Results show (Tab. 3, Fig. 4) that INT after treatment did not differ between treatment and control group (male $F=2.604$, $p=0.109$; female $F=0.007$, $p=0.934$). Though, time spent inside the nestbox was significantly longer in the first visits following the aromatic treatment in males ($F=17.043$, $p<0.0001$) and females ($F=21.193$, $p<0.0001$). In sum, females spent significantly longer periods in the nest than males under natural as well as experimental conditions (Tab. 4) as reflected in significantly longer hesitation times after moss treatment ($F=27.832$; $p<0.0001$) but also aromatic treatment ($F=57.737$; $p<0.0001$).

Due to small sample sizes results for female INT before as well as after treatment in the moss control group was severely influenced by outliers as illustrated in Figure 4. Distinct females stayed disproportionately long (max. 240 s) inside the nestbox during observation period, causing a biasing increase of mean INT. Therefore, all results including female INT of the moss control group have to be examined critically. Detrimental effects become apparent in the fact that time spent inside the nestbox by females was already influenced by treatment and visit order preliminary to treatment application (Tab. 3).

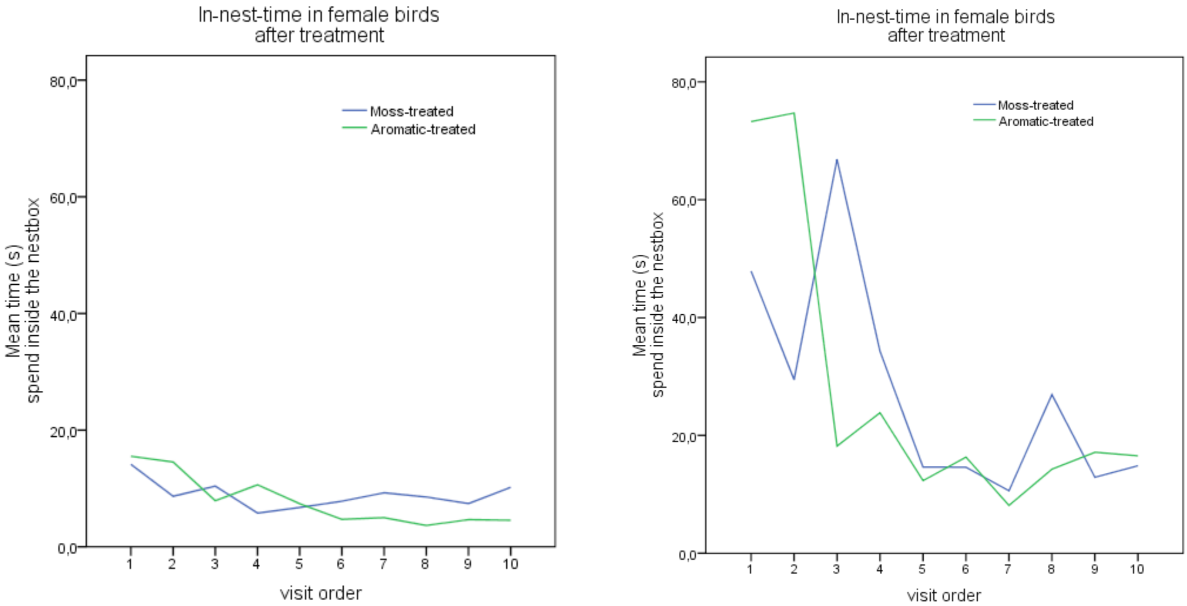

Fig. 4 Mean times (s) spend in the nestbox by adult birds over 10 successive visits in moss-treated control nests and aromatic-treated nests.

When comparing INT (Tab. 5) of male birds under natural and experimental conditions, times remain equal in moss-treated nests ($F=0.841$, $p=0.361$) as well as aromatic-treated nests ($F=3.408$; $p=0.067$). Differing results arise in female birds since INT did not differ under natural and experimental conditions in moss-treated nests ($F=0.363$; $p=0.548$) but varied significantly in aromatic-treated nests ($F=13.186$; $p<0.0001$). Variation occurs due to the mentioned outliers. Additionally INT in both sexes was significantly longer in first visits after aromatic treatment, reflected also in a variation of Spearman's rank correlation coefficient as given in Table 6.

Tab. 6 Correlation between latency and visit order, and in-nest-times and visit order as reflected in Spearman's rank correlation coefficient (r). Coefficients for comparison between treatment groups, sexes and analysis before and after

		Latency-times				In-nest-times			
		Male		Female		Male		Female	
		r	p	r	p	r	p	r	p
Experimental group	before treatment	-0.094	0.376	0.031	0.770	0.064	0.551	-0.191	0.071
	after treatment	-0.552	<0.0001	-0.531	<0.0001	-0.510	<0.0001	-0.339	0.001
Control group	before treatment	-0.143	0.204	-0.040	0.724	-0.127	0.294	-0.208	0.049
	after treatment	-0.072	0.525	-0.071	0.534	-0.025	0.829	-0.254	0.023

4. Discussion

In the last decades more and more experimental evidence arouse for the existence of olfactory abilities in different species of passerines. Comprehensive studies on Corsican blue tits demonstrate that both sexes are receptive to volatile odors (Petit *et al.* 2002; Mennerat, 2008; Mennerat *et al*., 2009) emitted by fresh aromatic plant species used for nest maintenance; a similar effect was demonstrated in European starlings by Clark & Mason in the late 1980s. Encouraged by these findings this study aimed to examine olfactory abilities in a non-greenery using passerine, the great tit. Since latency increased significantly after odor manipulation of the nest in both sexes, results maintain evidence that great tits are sensitive to volatile chemical cues, and adjust their behavior in response to the unknown odor. This adds further experimental evidence for the existence of olfactory abilities in another species of the order Passeriformes and supports the notion that even birds with small olfactory tissues are able to use olfaction. Hence great tits potentially join the line of passerine birds with assumed olfactory capacities like blue tits, European starlings (Clark & Mason, 1980) or collared flycatchers.

Parallel to this investigation two experiments were conducted to examine the role of olfaction in blue tits, which are noted to use olfactory cues in the context of aromatic plant acquisition for nest maintenance (e.g. Petit *et al*., 2002). By considering the current state of research in this field, new methodological approaches were tested to determine one of the major questions in this context that still remains unsolved. The functional biological significance of aromatic plant use in nest building remains speculation, although controversial results of different studies indicate that chemical compounds of distinct plant species reduce parasite load (LaFuma *et al.*, 2001; Wimberger, 1984) or bacteria (Mennerat, 2009) on nestlings, and have fungicidal (Adam *et al*., 1998) or anti-microbial (e.g. Hanamanthagouda *et al.*, 2010) functions.

A first experiment focused on a proposed potential significance of aromatic plants as predator repellents by testing the selectivity of blue tits in odor dependent choice of nesting material. Results show that birds confronted with experimentally scented nesting material, did not exhibit an odor dependent preference, since withdrawal quantity of dog's hair remained equal in all experimental groups. Lack of differences in withdrawal quantities is possibly due to inadvertent influence of other bird species that withdrew nesting material apart of blue tits. Hence, no conclusion on potential role of distinct nest odors in predator avoidance is to be made, although the experiment showed that birds accepted the tested experimental setup. For further investigation it is essential to repeat the experiment with a larger number of birds and presumably test the effect of olfactory stimuli used for nest maintenance on common predators in cavity-breeding bird species. Additionally, the experimental setting has to be adjusted to prevent detrimental interactions of other bird species using the experimental nesting material.

The second investigation preliminarily to the actual experiment focused on the role of aromatic nest odor composition on long-time parental investment. In their 'Potpourri hypothesis' Lambrechts & Dos Santos (2000) propose, that a complex bouquet of different aromatic herbs increases the repellent effect against various parasites or pathogens (Lambrechts & Dos Santos, 2000). This effect possibly contributes positively to nestling health since physical stress by parasite infestation is considerably reduced. One could presume that an increase of offspring fitness coincides with the extent of parental investment, since additional costs for foraging are compensated by raised survival probability in nestlings. Hence, it was expected that variations in nest odor bouquet, if somehow contributing to nestling fitness, elicits an alteration in male investment as reflected in variations in feeding intervals of the male bird. Results failed to show a significant long-term change of feeding intervals, although in all experimental groups a slight increase of time elapsed between intervals on the second day and a decrease on the third day of trials was detectable. This effect possibly occurs due to habituation processes, since a change in nest odor composition presumably stimulates cautious behavior in adult birds approaching the nestbox, which decreases with time elapsed. Hence feeding intervals of the third day approximated behavior under natural conditions. In the interpretation of results deteriorating weather conditions over trial period have to be considered as detrimental influencing parameter on feeding intervals. For significant conclusions on long-time effects of parental investment it is necessary to increase sampling size.

A recent study of Zelenitsky *et al.* (2011) reveals new astonishing evidence that contradicts the generally accepted theory of olfactory bulb reduction during avian evolution. It has been believed that the acquisition of sophisticated visual, auditive and proprioceptive skills essential for flight has been accompanied by a decline of olfactory abilities in birds. In their work, examining the olfactory bulb sizes of modern bird species and their theropod ancestors, Zelenitsky *et al.* showed that capabilities of odor detection actually increased during avian evolution, deducing that the sense of smell was of greater relevance than originally thought. A reduction of relative olfactory bulb size is first to be noticed in distinctive derived taxa of higher neognaths (Neoaves), and achieves maximum extent in the order of Passeriformes (Zelenitsky *et al.*, 2011). The authors speculate that olfactory abilities became redundant when passerines sophisticated their cognitive abilities. Though it is known that, in contrast to most other bird orders, there is no correlation between size of the olfactory bulb and function of the olfactory sense in passerines (Clark *et al.*, 1993).

Now that new experimental evidence demonstrates the use of olfaction in different species of Passeriformes, one has to speculate about the ecological mechanisms for why the olfactory sense retained its significance in passerine evolution. Since effect of aromatic volatiles on bird behavior was recognized in the context of nesting and breeding, it is assumed that the aromatic nest odor composition is of major significance in this context. Results suggest that birds were capable of detecting the artificial odor even before entering the nestbox and modified their behavior according to the unfamiliar aromatic environment. Birds of both sexes significantly delayed their entry to the nestbox in the first visits following the aromatic treatment or completely refused to enter the cavity by perching on the box or departing immediately. Withdrawal from the nestbox was always followed by repeated approaching attempts and was, after a maximum of 5 unsuccessful visits in a male and 7 in a female bird, concluded by a successful entry to the cavity. One could speculate that these specific behavioral responses in adult birds to a change in nest odor composition by either increasing latency before entering the cavity or repeating approach and withdrawal attempts somehow contribute to a vitally important compound of bird behavior, predator avoidance (Amo *et* *al.*, 2008). For secondary cavity-breeding species like blue tits the use of the olfactory sense for predator recognition could be inevitable. Different rodents (e.g squirrels or mice), competing bird species or even snakes, e.g. the Aesculapian snake (*Elaphe longissima*), presumably account for a majority of losses in clutches and nestlings and are at least partially able to enter a nest or nestbox (Walankiewicz, 2002). Since visual predator recognition from outside the nest cavity is limited a change in nest odor composition could therefore be a valuable clue for risk assessment before entering the nestbox. Birds precociously aware of predation risk, could adopt in a cautious behavior and circumvent an encounter with undesirable consequences. Concurrent are the results of Amo *et al.* (2008), who found that blue tits were able to detect real predator odor inside the nest and showed antipredatory behavior by delaying their entry to the cavity, coinciding with a decrease of time spend inside the box.

Results of this study failed to show a significant long-time effect of nest odor manipulation on times spent inside the nestbox. Though female birds spent significantly longer time spans inside the nestbox than males indicating differences in nest or nestling maintenance between sexes. These findings are consistent with Christe *et al.* (1996) who examined the effects of ectoparasites of offspring on parental behavior in great tits and found that only female birds engaged in sanitation activity during their stay in nest and therefore actively increased time spend in the cavity. This gives rise to the idea that females additionally prolonged their stay in the nest in the first two visits following an experimental treatment for intensified sanitation activities, since an unfamiliar disturbing factor, e.g. aromatic moss, was applied artificially. Hence results exhibit that visit order also had an impact on INT after odor manipulation in males (Tab. 3), indicating that INT was marginally longer in the first few visits. Possible assertion can be made when contemplating data for latency before entering. A significant effect of artificial nest odor manipulation as reflected in increased latency was strongest in the first visits following the aromatic treatment and decreased over successive approaches, suggesting that blue tits quickly habituated to the new aromatic environment inside their nests. Presumably accounting for drastically prolonged latency, but also increased INT in the first approaches is ineluctable open-field contact with the observer, eliciting avoidance behavior in birds similar to a predatory encounter. Though, observer effects are constant over trials and are therefore negligible for result interpretation. In both sexes the behavioral response constantly decreased with each approach and approximated natural magnitude by the end of observation. This seems to be a necessary mechanism, since deletion of foraging sorties and consequently decrease of food supply for offspring would affect nestling growth and survival probability negatively.

From the very beginning food limitation is an important factor in a bird's life, determining not only reproductive success but also individual survival (Martin, 1987). It seems plausible that the sense of smell somehow plays a role in foraging, since the availability of an additional sense would provide its owner with a competitive advantage over other species, eventually in food location and acquisition. Benefits in foraging coinciding with increased survival probabilities may have increased selective pressures for olfactory abilities in avian evolution. If great tits, which are native in Europe and reach high abundances in forests of moderate climates, are capable of using olfactory cues in the context of foraging, this may bring the necessary advantages in food competition. Enhanced olfactory prowess in the context of food location has already been demonstrated in kiwis (*Apteryx australis*), which are able to detect food by smell even at night (Wenzel, 1968), and is especially well studied in different species of Procellariiformes. These tube-nosed seabirds rather depend on olfactory than visual stimuli and use volatile olfactory cues to detect prey over the open sea. It has also been shown that some species are able to distinguish familiar birds by scent and relocate individual burrows by identifying the characteristic olfactory signature (e.g. Nevitt, 2008). Interestingly results of different behavioral studies on olfactory abilities in domestic chicken show that chicks are attracted to odors with which they have been reared, even in unfamiliar environments (Jones & Carmichael, 1999). Consistent are findings of Casper & Krause (2010), who showed that young zebra finch (*Taeniopygia guttata*) fledgelings, were attracted to the odor of their own nest and showed a preference over foreign nest odors (Casper & Krause, 2010). One could assume that even in residential great tits, olfactory cues play a role in small-scale orientation in familiar environments in adult birds as well as nestlings. However, it is premature to propose sophisticated olfactory skills in passerines similar to those of procellariiforme species. Nevertheless there is no lack of conceivable application possibilities of an olfactory sense in a bird's life. Exciting future investigations could for example focus on the role of olfaction in nest location, or even parent-offspring interactions, as demonstrated in ring doves (Cohen, 1981; as reviewed by Balthazar & Taziaux, 2009).

So far, the suggested biological meaning of the olfactory sense in great tits remains hypothetical. Further studies examining (1) whether great tits are able to discriminate between different odors, and furthermore (2) have the ability to recognize odors associated positively (e.g. food) and odors with detrimental relevance (e.g. predators) would be useful in addressing this issue. Finally, much more committed scientific research is required to identify the mechanisms underlying olfactory abilities in passerine birds.

5. References

Ache, B.W., Young, J.M. (2005): Olfaction: diverse species, conserved principles. Neuron 48, 417-430.

Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T., Arsenakis, M. (1998): Antifungal activities of Ori*ganum vulgare* subsp. *hirtum*, *Mentha spicata, Lavandula angustifolia,* and S*alvia fruticosa* essential oils against human pathogenic fungi. Journal of Agricultural and Food Chemistry 46, 1739-1745.

Amo, L., Galván, I., Tomás, G., Sanz, J.J. (2008): Predator odour recognition and avoidance in a songbird. Functional Ecology 22, 289-293.

Balthazart, J., Taziaux, M. (2009): The underestimated role of olfaction in avian reproduction? Behavioural Brain Research 200, 248-259.

Bang, B.G., Cobb, S. (1968): The size of the olfactory bulb in 108 species of birds. The Auk 85, 55-61.

Bang, B.G. (1971): Functional anatomy of the olfactory system in 23 orders of birds. Acta Anatomica (Suppl.) 58, 1-76.

Bonadonna, F., Cunningham, G.B., Jouventin, P., Hesters, F., Nevitt, G.A. (2003): Evidence for nest-odour recognition in two species of diving petrel. The Journal of Experimental Biology 206, 3719-3722.

Bonadonna, F., Nevitt, G.A. (2011): Partner-specific odor recognition in an Antarctic seabird. Science 306, 835.

Buitron, D., Nuechterlein, G.L. (1985): Experiments on olfactory detection of food caches by black-billded magpies. The Condor 87, 92-95.

Casper, B.A., Krause, E.T. (2010): Odour-based nest recognition in the zebra finch (*Taeniopygia guttata*) a colony-breeding songbird. Biology letters 7 (2), 184-186.

Christe, P., Richner, H., Oppliger, A. (1996): Of great tits and fleas: sleep baby sleep.... Animal Behaviour 52, 1087-1092.

Clark, L., Avilova, K.V., Bean, N.J. (1993): Odor thresholds in passerines. Comparative Biochemistry and Physiology Part A: Physiology 104 (2), 305-312.

Clark, L., Mason, J.R. (1987): Olfactory discrimination of plant volatiles by the European starling. Animal Behaviour 35 (1), 227-235.

Clark, L., Shivik, J. (2002): Aerosolized essential oils and individual natural product compounds as brown treesnake repellents. Pest Management Science 58, 775-783.

Cohen, J. (1981): Olfaction and parental behavior in ring doves. Biochemical Systematics and Ecology 9 (4), 351-354.

Gwinner, H., Berger, S. (2006): Parasite defence in birds: the role of volatiles. Acta Zoologica Sinica 52, 280-283.

Hagelin, J.C. (2004): Short communication. Observations on the olfactory ability of the Kakapo *Strigops habroptilus*, the critically endangered parrot of New Zealand. IBIS The International Journal of Avian Science 146 (1), 161-164.

Hanamanthagouda, M.S., Kakkalameli, S. B., Naik, P.M., Nagella, P., Seetharamareddy, H.R., Murthy, H.N. (2010): Essential oils of *Lavandula bipinnata* and their antimicrobial activities. Food Chemistry 118, 836-839.

Jones, R.B, Carmichael, N.L. (1999): Domestic chicks are attracted to a familiar odorant in a novel test situation: a brief report. Applied Animal Behaviour Science 61, 351-356.

LaFuma, L., Lambrechts, M.M., Raymond, M. (2001): Aromatic plants in birds nests as a protection against blood-sucking flying insects? Behavioural Processes 56, 113-120.

Lambrechts, M.M., Dos Santos, A. (2000): Aromatic herbs in Corsican blue tit nests: The 'Potpourri' hypothesis. Acta Oecologica 21 (3), 175-178.

Lambrechts, M.M, Hossaert-McKey, M. (2006): Olfaction, volatile compounds and reproduction in brids. Acta Zoologica Sinica 52, 284-287.

Lledo, P.-M., Gheusi, G., Vincent, J.-D. (2005): Information processing in the mammalian olfactory system. Physiological Reviews 85 (1), 281-317.

Martin, T.E. (1987): Food as a limit on breeding birds: a life-history perspective. Annual review of ecological systems 18, 453-487.

Mason, J.R., Clark, L. (2000): The chemical senses in birds. In: Sturkie's avian physiology. 5th edition, Causey Whittow, G. (Ed.), San Diego, Academic Press, 569-596.

Mennerat, A. (2008): Blue tits (*Cyanistes caeruleus*) respond to an experimental change in the aromatic plant odour composition of their nest. Behavioural processes 79 (3), 189-191.

Mennerat, A., Mirleau, P., Blondel, J., Perret, P., Lambrechts, M.M., Heeb, P. (2009): Aromatic plants in nests of the blue tit *Cyanistes caeruleus* protect chicks from bacteria. Oecologia 161, 849-855.

Mennerat, A., Perret, P., Lambrechts, M.M. (2009): Local individual preferences for nest materials in a passerine bird. PloS ONE 4 (4).

Nef, P. (1998): How we smell: The molecular and cellular bases of olfaction. News in Physiological Sciences 13 (1), 1-5.

Nevitt, G.A. (2008): Sensory ecology on the high seas: the odor world of the procellariiform seabirds. The Journal of Experimental Biology 211, 1708-1713.

Niimura, Y., Nei, M. (2006): Evolutionary dynamics of olfactory and other chemosensory receptor genes in vertebrates. Journal of human genetics 51, 505-517.

Papi, F., Fiore, L., Fiaschi, V., Benvenuti, S. (1971): The influence of olfactory never section on the homing capacity of carrier pigeons. Monitore zoologico italiano 5, 255-267.

Petit, C., Hossaert-McKey, M., Perret, P., Blondel, J., Lambrechts, M.M. (2002): Blue tits use selected plants and olfaction to maintain an aromatic environment for nestlings. Ecology Letters 5, 585-589.

Rieke, G.K., Wenzel, B.M. (1978): Forebrain projections of the pigeon olfactory bulb. Journal of Morphology 158 (1), 41-55.

Roper, T.J. (1999): Olfaction in birds. Advances in the Study of Behavior 28, 247-332.

Smith, S.A., Paselk, R.A. (1986): Olfactory sensitivity in the turkey vulture (*Cathartes aura*) to three carrion-associated odorants. The Auk 103 (3), 586-592.

Stager, K.E. (1967): Avian olfaction. American Zoologist 7 (3), 415-420.

Steiger, S.S., Fidler, A.E., Valcu, M., Kempenaers, B. (2008): Avian olfactory receptor gene repertoires: evidence for a well-developed sense of smell in birds? Proceedings of the Royal Society B 275, 2309-2317.

Walankiewicz, W. (2002): Breeding losses in the Collared Flycatcher *Ficedula albicollis* caused by best predators in the Bialowieza National Park (Poland). Acta Ornithologica 37 (1), 21-26.

Wenzel, B.M. (1968): Olfactory Prowess of the Kiwi. Nature 220, 1133-1134.

Wimberger, P.H. (1984): The use of green plant material in bird nests to avoid ectoparasites. The Auk 101, 615-618.

Zelenitsky, D.K., Therrien, F., Ridgely, R.C., McGee, A.R., Witmer, L.M. (2011): Evolution of olfaction in non-avian theropod dinosaurs and birds. Proceedings of the Royal Society B 278, 3625-3634.

The scent of something new. Evidence for olfactory abilities in a passerine bird, the collared flycatcher (*Ficedula albicollis*)

Abstract

Neurophysiological and behavioral studies on the avian olfactory system are rare but convincing, demonstrating well-developed olfactory capacities in distinct bird species. One order of the avian kingdom, the Passeriformes, has widely been excluded from this research due to historical misconceptions. Recent investigations however suggest that different species of passerines are able to perceive volatile cues in biologically relevant contexts. This study aimed to examine olfactory capacities in a long-distance migrant, the collared flycatcher (*Ficedula albicollis*), by manipulating nest odor composition and monitoring short-term behavioral responses. In a total sample of 17 nests of a wild population breeding in nestboxes, one group was exposed to an artificial odor (*Lavandula officinalis)* while the other acted as a control. A potential effect of treatment was measured as reflected in variations of latency before entering the nestbox or time spent inside the nest. Results maintain evidence that free-ranging collared flycatchers are sensitive to volatile compounds added artificially to the nest, since latencies before entering the nestboxes increased significantly after odor treatment in both sexes. We present possible assertions for specifics in results and discuss the potential role of olfaction in the life of a long-distant migrant.

1. Introduction

In a world of odors, the ability to detect volatile chemical substances by means of olfaction is an essential skill that mediates multiple interactions with the environment. Ubiquitous natural odors are important in a variety of contexts, e.g. foraging, communication, homing, individual recognition, mating, orientation, territorial marking or predator detection. Hence, olfaction is a primary important sense in many vertebrates, but even in most basal organisms the role of chemical sensitivity is undeniable (Ache & Young, 2005).

Even more surprising that birds were regarded to have little or no olfactory perception for decades, relying rather on vision and acoustics as major sensory modalities (as reviewed by Roper, 1999). This idea remained widely accepted until the late 1960s, when Bang and Cobb (1968) measured the relative olfactory bulb size in 108 different species of birds, presuming that an evolutionary enhancement of a part of the brain implies an upgrade in function (Bang & Cobb, 1968; Bang, 1971). Measurements showed that the olfactory bulb took up more than 30% in the brains of different tube-nosed seabirds and only 3% in a passerine songbird. Olfactory sense seemed to be, following the conclusion size entails function, of primary importance in species with great olfactory bulb ratios over 28% (Buitron & Nuechterlein, 1985) and is, based on little relative olfactory bulb ratios under 9,7%, relatively unimportant in other groups like the Passeriformes (Bang & Cobb, 1968). This interpretation is consistent with findings of various field experiments, which show that species known to posses large olfactory bulbs, like Procellariiformes (e.g. Bonadonna *et al*., 2003). or kiwis (Wenzel, 1968) use olfaction in distinct behavioral contexts.

Nowadays, a steadily increasing amount of research is done to examine the olfactory sense and its biological relevance in birds, bringing evidence for high olfactory sensitivity and well-developed discrimination capabilities in distinct species. Based on comprehensive neurophysiological and anatomical studies the development of the avian olfactory sense is actually believed to be almost on the same level with that in mammals (Mason & Clark, 2000), since organization of the olfactory epithelium and structural details of the olfactory bulbs in birds show similarities to those found in mammals and other vertebrates (Balthazar & Taziaux, 2009). Additionally, electrophysiological studies of Rieke & Wenzel 1978 (as reviewed by Balthazar & Taziaux, 2009) on pigeon brains demonstrated that electrical signals, which are induced when encountering an odorous stimulus, are transmitted to telencephalic loci and further on to different behavior-relevant brain areas that are potentially able to process the received information. Accurate functioning of this mechanism is experimentally assured, since different studies under laboratory as well as natural conditions supply detailed evidence, that the presence of an olfactory stimulus elicits behavioral responses in a variety of situations. Particularly well studied (e.g. for reviews, see Roper, 1999; Balthazart & Taziaux, 2009) are foraging in kiwis (Wenzel, 1968), kakapos (Hagelin, 2004) and new world vultures (Stager, 1967; Smith & Paselk, 1986), nest and individual recognition in tube-nosed seabirds (Bonadonna *et al.*, 2003; Bonadonna & Nevitt, 2011; Nevitt, 2008), orientation in homing pigeons (Papi *et al.*, 1971) and preference of familiar odors in zebra finch fledglings (Casper &

Krause, 2010).

Although the use of olfaction is no longer questioned its relevance in one order of the avian kingdom, the Passeriformes, which includes more than half of all bird species known (Martin, 1986), remains poorly investigated due to historical reasons. Literature pertaining to the olfactory sense in passerines is scarce but convincing, pointing to the fact that odorous stimuli play a role in initiation of spontaneous behaviors in different species. In 1987 Clark & Mason found that European starlings (*Sturnus vulgaris*) are able to use volatile cues to discriminate between fresh plant material, since starlings, like few other passerines, incorporate fresh plant fragments preferably rich in volatile compounds (Gwinner & Berger, 2006) into their nests during nesting period (Wimberger, 1984). In the following years these findings cleared the way for several other studies investigating the use of volatile compounds in nest building and maintenance primarily in starlings and another member of the passerine family, the blue tit. On Corsica female blue tits (*Cyanistes caeruleus*) daily add fresh aromatic herbaceous plants to their nests (Lambrechts & Dos Santos, 2000) and are able to discriminate between different plant volatile compounds as well as perceive variations in odor concentrations (Petit *et al*., 2002). Recent findings (Mennerat, 2008) under semi-natural conditions suggest that blue tits are capable of detecting a change in the aromatic composition from outside the nest cavity. After experimental manipulation by adding aromatic plant fragments to the nest, birds of both sexes showed a significant increase in latency before entering the cavity. Although the basal mechanisms underlying this behavioral response remain speculation, one can assume that olfaction is of primary importance even in 'small-bulbed' Passeriformes.

In the breeding season of 2011 a set of experiments was conducted to examine the role of olfaction in three species of passerines, only one of them, the blue tit, known to incorporate fresh plant material into their nests. Preliminary results bring evidence that great tits (*Parus major*), although naturally not using green herbaceous nest material, are sensitive to volatile compounds added artificially to the nestbox. Encouraged by these findings and the results of Mennerat (2008) this study aimed to examine the existence of olfactory abilities in another non-greenery using passerine, the collared flycatcher (*Ficedula albicollis*). These birds from the family of Muscicapidae are long-distance migrants, breeding in southeast Europe and wintering in trans-Saharan Africa. In constantly changing environments one could presume the olfactory sense to be an indispensable and ubiquitous source of information in various environmental interactions.

Proposing collared flycatchers as suitable experimental subjects an experiment was designed to investigate the role of olfaction in a free-ranging population of these passerines by manipulating nest odor composition and monitoring short-term behavioral responses. Possible behavioral effects of odor treatment are expected to reflect in a variation of latency before entering the nest cavity or variations in times spend inside the nestbox. It is estimated that after odor manipulation of the nest adult collared flycatchers of both sexes, if receptive to odors, will engage in cautious behavior either by elongating latency before entering or time spent in the nestbox. Results and feasible mechanisms for these effects will be critically discussed with regard to biological relevance of the olfactory sense in the life of a long-distance migrant.

2. Materials and Methods

Experiments were conducted in the breeding season of 2011 in the north-eastern part of the Wienerwald in Vienna (Austria) where nestboxes are accepted for breeding by collared flycatchers (*Ficedula albicollis*). The long distance-migrants reach their breeding grounds by mid April, mating, egg deposition and breeding initiates immediately after arrival and continues until mid June. Clutch sizes range from five to eight. From the onset of nest building the nestboxes were monitored every day to examine the progress of nest construction, the onset of egg laying, hatching date and number and constitution of the nestlings until fledging.

To achieve an adequate sample size, trials were carried out in the area of Kolbeterberg and Buchberg on a total sample of 17 nests (days 10-12 post hatching), assigned randomly to two experimental groups: one group was exposed to an experimental odor (n=8) while the other acted as a control (n=9). The aromatic treatment consisted of 1 drop of essential oil (Primavera Life GmbH) with lavender (*Lavandula officinalis*) as main component, the control treatment of 1 drop of water, added to 2g of moss. Nests of the treatment group were provided with fragranted moss, nests of the control group with moisture moss only, which was hidden in the rear part of the nest to avoid premature removal by adult birds or direct contact of nestlings with the odor. Application of essential oils seemed appropriate since dosage can be standardized and testing conditions are replicable. Therefore the amount of odorous substances added was constant in each nest of the treatment group. All experimentally added materials were removed directly after the experiment.

Lavender is without known biological significance to the tested species, but is verified to be incorporated by Corsican blue tits (*Cyanistes caeruleus*) into their nests (Petit *et al.*, 2002), demonstrating a sensitivity of olfactory receptors to the component in this species. The obvious relevance of lavender as olfactory stimulus in a related biological context and lack of any discernible irritating components justifies the use in this experiment.

All observations were performed at approximately 20-30 m from the nestboxes by using a spotting scope and making use of natural shelters, e.g. trees, trunks or rocks. To keep disturbance to a minimum, nestboxes were not opened for experimental treatment until both parents had left the box and disappeared completely from the observer's sight. Recognition between sexes during the experiment was alleviated by the fact of sexualdimorphism in plumage coloration. In the breeding season males are mainly black with white patches, while females are of a typical brown coloration.

Behavioral observation initiated immediately after application of the treatment and consisted of recording (1) the number of unsuccessful visits of each parent before entering the nestbox, (2) latency (LT) or hesitation time of male and female before entering the nestbox and (3) the time spend in the nestbox (in-nest-time, INT) by each parent. Preliminary to the actual experiment, all nests were monitored without experimental influence to determine the natural behavior of birds. All variables were therefore measured under natural conditions before and experimental conditions after treatment. An unsuccessful visit occurred, when a bird approached the nestbox and left immediately without entering the cavity. Latency or hesitation time was defined as the time the bird was in direct physical contact with the nestbox without entering. Recording of INT and LT stopped after 12 successful visits, i.e. entrances to the nestbox for a feeding purpose, and was measured in seconds (s). Monitoring was performed by one single observer. Time (in seconds) was recorded with a stopwatch.

Prior to statistical analysis latencies and in-nest-times were log plus one transformed to approximate a normal distribution. An analysis of covariance (ANCOVA) was performed with LT and INT as dependent variable and visit order (visits 1-12) as covariat, to correct results from the influence of habituation. Treatment, sex and time of assessment (before/after treatment) were included as fixed factors. Results show possible differences in LT and INT before and after treatment, between sexes and treatment groups with respect to a distinct habituation. For achievement of improved comparability of LT and INT over successive visits linear regression models were applied and Spearman's rank correlation coefficient was employed as method for comparative analysis. All statistical analysis were done using SPSS (version 16.0) software.

3. Results

Latency before entering the nestbox increased significantly after treatment in birds of both sexes assigned to the aromatic treatment group (male $F=32.335$, $p<0.0001$; female $F=37.04$, $p<0.0001$) in comparison to the moss control treatment group. Results are given in Figure 1 and Table 1. This distinct effect was highest in the first few visits and decreased significantly over successive approaches in male ($F=31.01$; $p<0.0001$) and female ($F=26.99$, $p<0.0001$), reflected also in altered Spearman's rank correlation coefficients (Tab. 4).

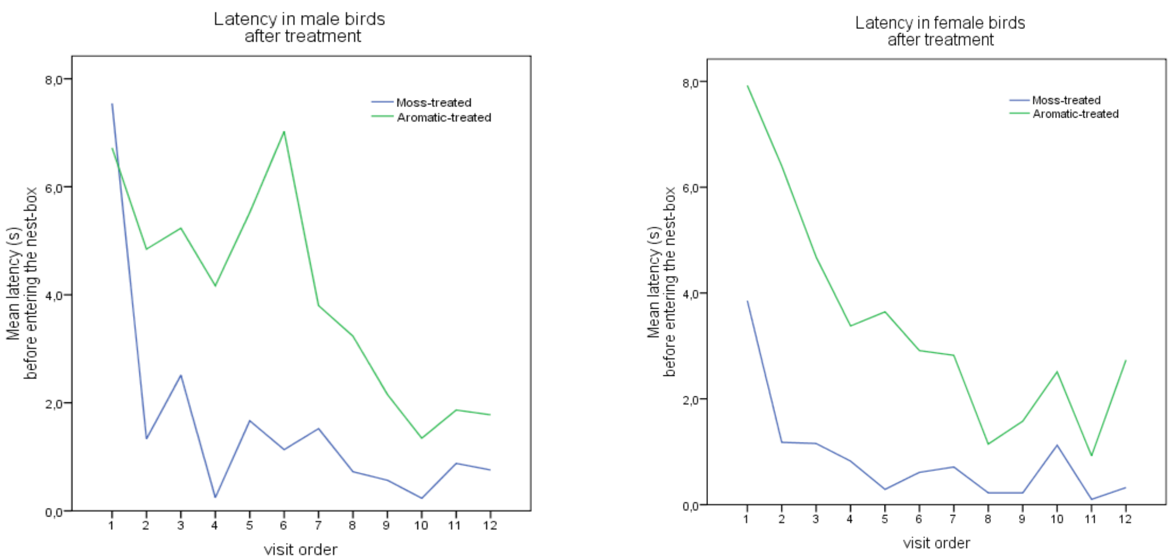

Fig. 1 Mean latency (s) before entering the nestbox in adult birds over 12 successive visits in moss-treated control nests and aromatic-treated nests. Hesitation times are significantly longer after aromatic treatment in both sexes.

Tab. 1 Effects of visit order and treatment, before and after experimental treatment on latency and times spend in the nestbox in male and female birds, as tested by analysis of covariance (ANCOVA).
d.f. … degrees of freedom

			Latency before entering				In-nest-times			
			Male		Female		Male		Female	
		d.f.	F	P	F	P	F	P	F	P
Before treatment	visit order	1	0.984	0.322	0.482	0.488	3.608	0.059	6.494	0.052
	treatment	1	0.376	0.540	0.091	0.762	1.984	0.160	3.409	0.066
After treatment	visit order	1	31.010	<0.0001	26.987	<0.0001	2.120	0.147	28.244	<0.0001
	treatment	1	32.335	<0.0001	37.041	<0.0001	10.073	0.002	0.350	0.555

Unsuccessful approaches of birds to the nestboxes were most frequently observed after odor treatment, with a mean number of 4.5 unsuccessful approaches in male and 3.1 in female. The behavior of approach and instant departure without entering the nest cavity only occurred in the time period immediately following treatment and reached a maximum of 11 unsuccessful visits in male and 10 in female. Natural approach behavior, e.g. approach followed by entry of the cavity for feeding purpose, was restored and maintained when the bird accomplished a first successive entry to the nestbox. Unsuccessful approaches could not be observed under natural conditions preceding the experiment and occurred only once in male, and twice in female birds after treatment in the moss control group.

Preliminary to treatment application, latencies were equal in treatment groups (Tab. 1) and sexes (Tab.2), indicating that hesitation times remain constant and approach behavior in male and female resembles under natural conditions. Behavioral response to an olfactory change in nestbox conditions, as reflected in hesitation time, did not differ between the sexes ($F=1.249$, $p=0.265$) but decreased significantly over successive visits ($F=31.724$, $p<0.0001$).

Tab. 2 Effects of visit order and sex, before and after experimental treatment on latencies and times spend in the nestbox in moss-treated and aromatic-treated nests, as tested by analysis of covariance (ANCOVA).
d.f. … degrees of freedom

			Latency before entering				In-nest-times			
			Moss-treated		Aromatic-treated		Moss-treated		Aromatic-treated	
		d.f.	F	p	F	p	F	P	F	P
Before treatment	visit order	1	0.000	0.992	0.158	0.692	0.266	0.606	0.029	0.866
	sex	1	2.224	0.107	0.372	0.543	87.215	<0.0001	32.061	<0.0001
After treatment	visit order	1	26.996	<0.0001	31.724	<0.0001	13.513	<0.0001	10.650	0.001
	sex	1	2.089	0.150	1.249	0.265	60.802	<0.0001	28.909	<0.0001

A direct comparison of latency before and after treatment (Tab. 3) shows that hesitation times after odor application were significantly longer than under natural conditions (male F=71.879; p<0.0001; female F=53.258, p<0.0001). However, birds assigned to the moss control group did not hesitate longer before entering the nest cavity after the experimental treatment, although behavioral modifications possibly due to observer effects immediately after moss-treatment, reflected in longer latency in the first visits (male F=9.951, p=0.002; female F=6.235, p=0.013), were detectable.

Tab. 3 Effects of visit order and time of assessment (before/after treatment), in moss-treated and aromatic-treated nests on latency and times spend in the nestbox in male and female birds, as tested by analysis of covariance (ANCOVA).
d.f. … degrees of freedom

			Latency before entering				In-nest-times			
			Male		Female		Male		Female	
		d.f.	F	p	F	p	F	P	F	P
Moss-treated	visit order	1	9.951	0.002	6.235	0.013	0.298	0.586	23.144	<0.0001
	before/after	1	3.008	0.084	3.631	0.058	0.009	0.926	0.363	0.547
Aromatic-treated	visit order	1	16.075	<0.0001	9.120	0.003	0.023	0.880	10.600	0.001
	before/after	1	71.879	<0.0001	53.258	<0.0001	2.926	0.089	2.792	0.096

Spearman's rank correlation test (Tab. 4) shows a significant negative correlation between latency-times and visit order after treatment in sexes and treatment groups, supporting the assumption that hesitation times were longest in the first few visits and decrease over successive approaches. With regard to coefficients observer effects, especially in the first visits immediately after treatment, become obvious.

When comparing INT of treatment and control group (Fig. 2), time spent inside the nestbox was affected by odor treatment only in males (F=10.073, p=0.002). In females INT of treatment and control group did not differ (F=0.350; p=0.555), but was significantly longer in the first visits (F=28.244; p<0.0001). In sum, females spent significantly longer periods in the nest than males under natural (F=87.215; p<0.0001) as well as experimental conditions, as reflected in significantly longer hesitation times after moss treatment (F=60.802; p<0.0001) but also aromatic treatment (F=28.909; p<0.0001).

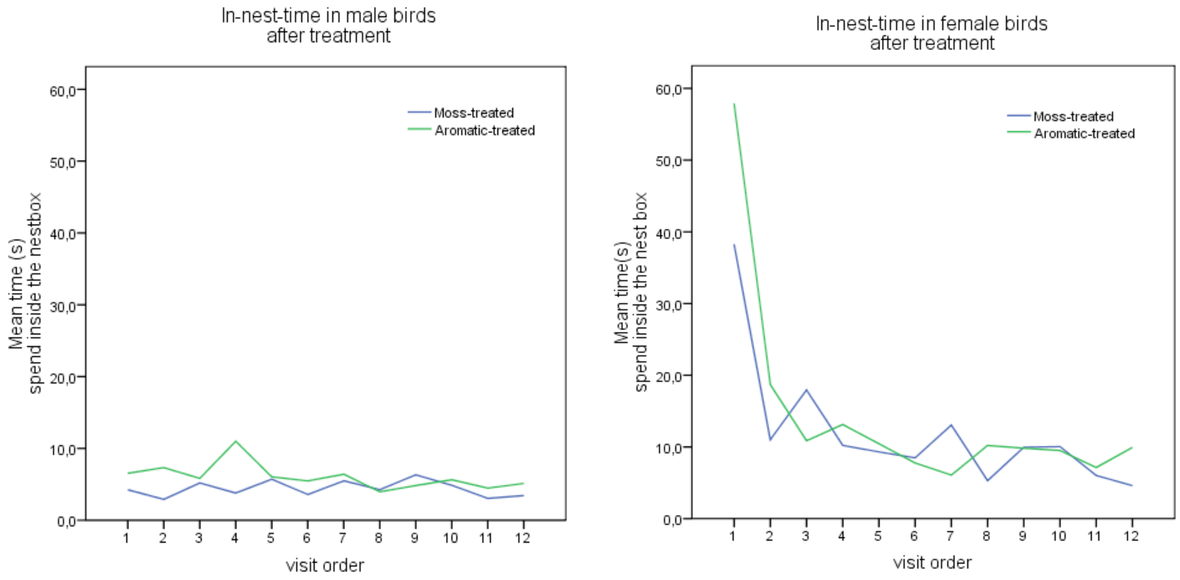

Fig. 2 Mean times (s) spend in the nestbox by adult birds over 12 successive visits in moss-treated control nests and aromatic-treated nests. In-nest-times vary with treatment only in males but not in females.

In direct comparison of INT of males under natural and experimental conditions, times remain equal in moss-treated nests (F=0.009; p=0.926), but also in odor-treated nests, contradicting an effect of aromatic odor manipulation on INT. Similar results arise in female birds, since INT did not differ under natural and experimental conditions in moss-treated (F=0.363; p=0.547) or odor-treated nests (F=2.792; p=0.096).

Hence INT of females again was significantly longer in first visits after treatment, reflected in a variation of Spearman's rank correlation coefficient as given in Table 4.

Tab. 4 Correlation between latency-times and visit order, and in-nest-times and visit order as reflected in Spearman's rank correlation coefficient (r). Coefficients for comparison between treatment groups, sexes and analysis before and after treatment are given.

		Latency				In-nest-times			
		Male		Female		Male		Female	
		r	P	r	P	r	P	r	P
Experimental group	before treatment	-0.065	0.507	-0.001	0.991	0.085	0.379	-0.153	0.115
	after treatment	-0.389	<0.0001	-0.324	0.001	-0.128	0.186	-0.220	0.022
Control group	before treatment	-0.049	0.617	0.069	0.484	0.155	0.109	-0.165	0.091
	after treatment	-0.254	0.008	-0.319	0.001	-0.077	0.426	-0.369	<0.0001

4. Discussion

Parallel to this investigation a set of experiments was conducted to examine the role of olfaction in three different species of passerine birds. Thereof one study analyzed the sensitivity to volatile compounds in residential great tits, whereas the present study focused on the olfactory abilities of a migratory passerine, the collared flycatcher, by documenting an alteration in behavior when adding odorous substances artificially to the nest. Both experiments resemble in methods but also in results: immediately after the odor treatment birds of both species and sexes showed a significant increase in latency to enter the nestbox. This adds further experimental evidence for the existence of olfactory abilities in another species of the order Passeriformes and supports the notion that even birds with a small olfactory bulb are able to use olfaction. Hence collared flycatchers as well as great tits potentially join the line of passerine birds with assured olfactory capacities like blue tits (Petit *et al*. 2002; Mennerat *et al*., 2004) or European starlings (Clark & Mason, 1987).

Results show that a significant effect of artificial nest odor manipulation as reflected in increased latency was strongest in the first visits following the aromatic treatment and decreased over successive approaches, indicating that collared flycatchers quickly habituated to the new aromatic environment inside their nests. Presumably accounting for drastically prolonged latency in the first approaches is ineluctable open-field contact with the observer, eliciting avoidance behavior in birds similar to a predatory encounter. Though, observer effects are constant over trials and are therefore negligible for result interpretation. In both sexes the behavioral response constantly decreased with each approach and approximated natural magnitude by the end of observation. It seems likely that nest odor manipulation has no lasting impact on feeding behavior of adult birds, for good reason. Long-term maintenance of cautious behavior would affect nestling growth and condition negatively, since feeding intervals are delayed and adequate supply for offspring is inhibited.

Interestingly, female birds spend significantly longer time spans inside the nestbox than males indicating differences in time management between sexes. These findings are consistent with Christe *et al.* (1996) who examined the effects of ectoparasites of offspring on parental behavior in great tits and found that only female birds engaged in sanitation activity during their stay in nest and therefore actively increased time spend in the cavity. This gives rise to the idea that females additionally prolonged their stay in the nest in the first two visits following an experimental treatment for intensified sanitation activities, since an unfamiliar disturbing factor, e.g. aromatic moss, was applied artificially. Hurtrez-Boussès *et al.* (2000), who showed a similar behavior in Mediterranean blue tits, speculate that an increase of INT for sanitation purpose coincides with a decrease in time female birds can invest in foraging or self-maintenance. Although the existence of this behavior and the obviously deriving trade-off between nest sanitation and self-maintenance has been verified in different studies its distinct purpose and relevance for nestling fitness remains unknown.

Collared flycatchers from the family of Muscicapidae are long distance migrants breeding in the moderate climate of southeast Europe with wintering quarters in sub-Saharan Africa. It seems plausible that birds, constantly facing variable environments, developed sophisticated sensory abilities which enable and alleviate life in differing ecological conditions. Now that experimental evidence points to the fact that sensory capabilities of collared flycatchers, apart from vision and acoustics, include the ability of olfaction one has to speculate about the biological relevance of remote chemoreception in the life of a long-distance migrant. Since an olfactory impact on bird behavior was recognized in the context of nesting and breeding, it is assumed that the aromatic nest odor composition is of major significance in this context. Results suggest that birds were capable of detecting the artificial odor even before entering the nestbox and modified their behavior according to the unfamiliar aromatic environment. Birds of both sexes significantly delayed their entry to the nestbox in the first visits following the aromatic treatment or completely refused to enter the cavity by perching on the box or departing immediately. Withdrawal from the nestbox was always followed by repeated approaching attempts and was, after a maximum of 11 unsuccessful visits in a male and 10 in a female bird, concluded by a successful entry to the cavity. It is known that birds engage in aversive behavior in response to unknown odors in a variety of biological contexts (Jones *et al.*, 2002). One could speculate that these specific behavioral responses in adult birds to a change in nest odor composition, by either increasing latency before entering the cavity or repeating approach and withdrawal attempts, somehow contribute to a vitally important compound of bird behavior, predator avoidance (Amo *et al.*, 2008).

In the study areas nestboxes are established and accepted for breeding for many years, providing potential predators an easily accessible and annually constant source of food (Czeszczewik *et al.*, 1999). For secondary cavity breeding bird species like collared flycatchers the use of the olfactory sense for predator recognition could be inevitable. Since visual detection inside the cavity is limited, chemical cues could facilitate hazard assessment even without entering the nestbox. Birds precociously aware of predation risk could adopt in a cautious behavior and circumvent an encounter with undesirable consequences. Concurrent are the results of Amo *et al.* (2008), who examined the behavioral response of adult blue tits to predator odor added artificially to the nestboxes. They found that birds were able to detect the odor cues and showed antipredatory behavior by delaying their entry to the cavity coinciding with a decrease of time spend inside the nestbox. Similar results of Fluck *et al.* (1996) suppose predator scent avoidance reactions in domestic chicks tested with cat odor, although a significant effect could only be demonstrated in 7-day-old chicken.

Contradictory to the findings of Amo *et al.* (2008) results of this study failed to show a clear and significant effect of odor treatment on the time spend inside the nestbox. In direct comparison of treatment group and moss control group, time spend in the nestbox was affected by odor treatment only in males. This effect vanished when comparing INT under natural conditions before and experimental conditions after treatment. It seems likely that, due to small sample sizes, data for INT is disproportionately high affected by outliers causing discrepancies in results. For any further investigation and a significant conclusion greater sample sizes are needed. Since time spent inside the nestbox in females did neither differ between natural and experimental conditions nor between the two experimental groups, INT are assumed to remain constant irrespective of odor treatment. These results are conclusive with Mennerat (2008), who also failed to show a significant impact of odor manipulation on times spend in the nestbox by blue tits.

In the experiment of Amo *et al.* (2008) real predator odor of mustelids was applied to nestboxes of blue tits, eliciting aversive behavior and a decrease in time spend inside the nests. In the control groups treated with water or quail odor only, none of the mentioned effects were measurable. One could assume that birds, when exposed to predator odor, reduce in-nest-times in order to minimize potential predation risk and desist from adopting in this energetically detrimental behavior when detecting an unknown odor not associated with an imminently lurking risk. So far, the suggested biological meaning of nest odor in the context of predator recognition and avoidance in collared flycatchers remains hypothetical. Further studies examining (1) whether collared flycatchers are able to discriminate between different odors, and furthermore (2) have the ability to recognize odors indicating the presence of a predator and odors without detrimental relevance would be useful in addressing this issue. Based on personal observations one of the main threats to collared flycatcher offspring the Aesculapian snake (*Elaphe longissima*), but also rodents (e.g. squirrels, weasels, mice) or woodpeckers (Walankiewicz, 2002) could be seen as appropriate in this context, since these predators presumably account for a majority of losses in clutches and nestlings. Assessment of predation risk by scent could be of great advantage for a passerine in the context of everyday life, e.g. foraging, homing or mating.

When speculating about the role of the olfactory sense in a long-distance migrant one inevitably has to consider the controversial issue of avian olfactory orientation and navigation. Depending on ecological specializations and environmental restraints, mechanisms and sensory tools for long-distance navigation vary among avian species and taxa (Alerstam, 2006). Modern concepts propose, in short, that avian navigation requires combining multimodal sensory inputs and can be seen as system based on innate orientation tools (e.g. magnetic compass, internal clock) complemented by other mechanisms (e.g. sun compass, landmarks, star compass, geophysical factors, polarized light) obtained in respective ontogentic learning processes (Wiltschko & Wiltschko, 2003; Alerstam, 2006). Following Kramer's 'map-and-compass' model (Kramer, 1953), avian navigation seems to be a two-step process, by first establishing the own geographic position relative to the goal with the help of an external reference and determining the theoretical flight direction as compass course. In the second step this information is converted into an actual homing direction by use of a compass (Wiltschko & Wiltschko, 2003; Nehmzow & Wiltschko, 2001). This model has recently been adjusted to include modern aspects of avian navigation, e.g. route integration (Wiltschko & Wiltschko, 2003). With regard to environmental specifications each species utilizes a distinct set of compass features as navigational system (Grocott, 2003). Due to conflicting and contradictory evidence the basal mechanisms of avian navigation still remain unknown. Especially when it comes to olfaction, presumably the most controversial issue in avian navigation, opinions are diverging and mostly skeptical (for detailed review, see Wiltschko & Wiltschko, 2003).

Studies began more than three decades ago when 10 homing pigeons (*Columba livia*), experimentally deprived from their sense of smell, were released to analyze their homing abilities (Papi *et al*., 1971). Over the years an increasing number experiments dealt with the role of olfaction in avian navigation leading to the contemporary conclusion, as reviewed by Wallraff, one of the leading scientists in this field, 'that displaced homing pigeons, and most likely other birds as well, are able to navigate home by deducing positional information from atmospheric trace gases perceived by olfaction' (Wallraff, 2004).

This assumption remains hypothetical lacking adequate scientific evidence since most of the debate focuses on identifying a suitable biogenic volatile compound at appropriate concentrations that migrants could use for navigation (Nevitt, 2008). Hence, facing the fact that recent studies bring evidence for a constantly rising number of bird species with assured olfactory abilities, one could speculate that the sensory input of an additional sense somehow contributes to the complex process of orientation and navigation. Berthold (2001) suggests that volatile atmospheric cues are utilized in combination with intrinsic compass-based programs, when navigating from breeding grounds to winter quarters. Hence, volatile signals are suspected rather to be used in small-scale navigation in proximity of the target area than in long-distance migration (Wallraff, 2004). Interestingly results of different behavioral studies on olfactory abilities in chicken show that chicks are attracted to odors with which they have been reared, even in unfamiliar environments (Jones & Carmichael, 1999). This ability was enhanced and refined in several species of petrels, who are known to relocate their nests by identifying the individual olfactory signature of their own nesting burrows (Bonadonna *et al*., 2003), even during dark nights. However, based on the current state of research it is premature to assume a somehow similar mechanism in homing of migrating birds. The mechanisms for avian long-distance navigation and the role of olfaction in this context remain hypothetical and will require committed research efforts in the future to fully understand the principles and complexities of migration.

In summary results of this study suggest the ability of collared flycatchers to perceive volatile substances by means of olfaction. For further investigation it is essential to repeat the experiment with a larger number of birds and presumably vary the type of the aromatic stimuli used. Finally, the basal biological mechanisms underlying the behavioral response to a change in nest odor composition still remain unknown, allowing speculations only about the relevance of an olfactory sense in long-distance migrants. Addressing these ambiguities could be the next challenging step towards a better understanding of the role of olfactory abilities in birds.

5. References

Ache, B.W., Young, J.M. (2005): Olfaction: diverse species, conserved principles. Neuron 48, 417-430.

Alerstam, T. (2006): Conflicting evidence about long-distance animal migration. Science 313, 791-794.

Amo, L., Galván, I., Tomás, G., Sanz, J.J. (2008): Predator odour recognition and avoidance in a songbird. Functional Ecology 22, 289-293.

Balthazart, J., Taziaux, M. (2009): The underestimated role of olfaction in avian reproduction? Behavioural Brain Research 200, 248-259.

Bang, B.G., Cobb, S. (1968): The size of the olfactory bulb in 108 species of birds. The Auk 85, 55-61.

Bang, B.G. (1971): Functional anatomy of the olfactory system in 23 orders of birds. Acta Anatomica (Suppl.) 58, 1-76.

Berthold, P. (2001): Bird migration. A general study. 2nd edition, Oxford, Oxford University Press, 1-238.

Bonadonna, F., Cunningham, G.B., Jouventin, P., Hesters, F., Nevitt, G.A. (2003): Evidence for nest-odour recognition in two species of diving petrel. The Journal of Experimental Biology 206, 3719-3722.

Bonadonna, F., Nevitt, G.A. (2011): Partner-specific odor recognition in an Antarctic seabird. Science 306, 835.

Buitron, D., Nuechterlein, G.L. (1985): Experiments on olfactory detection of food caches by black-billded magpies. The Condor 87, 92-95.

Casper, B.A., Krause, E.T. (2010): Odour-based nest recognition in the zebra finch (*Taeniopygia guttata*) a colony-breeding songbird. Biology letters 7 (2), 184-186.

Czeszczezewik, D., Walankiewicz, W., Mitrus, C., Nowakowski, W.K. (1999): Nest-box data on the Pied Flycatcher *Ficedula hypoleuca* may lead to erroneous generalizations. Vogelwelt 120: 49-153.

Christe, P., Richner, H., Oppliger, A. (1996): Of great tits and fleas: sleep baby sleep.... Animal Behaviour 52, 1087-1092.

Clark, L., Mason, J.R. (1987): Olfactory discrimination of plant volatiles by the European starling. Animal Behaviour 35 (1), 227-235.

Fluck, E., Hogg, S., Mabbutt, P.S., File, S.E. (1996): Behavioural and neurochemical responses of male and female chicks to cat odour. Pharmacology Biochemistry and Behavior 54 (1), 85-91.

Grocott, D.F.H. (2003): Maps in mind – How animals get home? The Journal of Navigation 56, 1-14.

Gwinner, H., Berger, S. (2006): Parasite defence in birds: the role of volatiles. Acta Zoologica Sinica 52, 280-283.

Hagelin, J.C. (2004): Short communication. Observations on the olfactory ability of the Kakapo *Strigops habroptilus*, the critically endangered parrot of New Zealand. IBIS The International Journal of Avian Science 146 (1), 161-164.

Hurtrez-Boussès, S., Renaud, F. (2000): Effects of ectoparasites of young on parents' behaviour in a Mediterranean population of Blue Tits. Journal of Avian Biology 31, 266-269.

Jones R.B., Carmichael, N.L. (1998): Domestic chicks are attracted to a familiar odorant in a novel test situation: a brief report. Short communication. Applied Animal Behaviour Science 62, 351-356.

Jones, R.B., Facchin, L., McCorquodale, C. (2002): Social dispersal by domestic chicks in a novel environment: reassuring properties of a familiar odourant. Animal Behaviour 63: 659-666.

Kramer, G. (1953): Wird die Sonnenhöhe bei der Heimfindeorientierung verwertet? Journal für Ornithologie 94, 201-219.

Lambrechts, M.M., Dos Santos, A. (2000): Aromatic herbs in Corsican blue tit nests: The 'Potpourri' hypothesis. Acta Oecologica 21 (3), 175-178.

Martin, G.R. (1986): The eye of a passeriform bird, the European starling (Sturnus vulgaris): eye movement amplitude, visual fields and schematic optics. Journal of Comparative Physiology A 159, 545-557.

Mason, J.R., Clark, L. (2000): The chemical senses in birds. In: Sturkie's avian physiology. 5th edition, Causey Whittow, G. (Ed.), San Diego, Academic Press, 569-596.

Mennerat, A., Bonadonna, F., Perret, P., Lambrechts, M.M. (2005): Olfactory conditioning experiments in a food-searching passerine bird in semi-natural conditions. Behavioural Processes 70, 264-270.

Mennerat, A. (2008): Blue tits (*Cyanistes caeruleus*) respond to an experimental change in the aromatic plant odour composition of their nest. Behavioural processes 79 (3), 189-191.

Nehmzow, U., Wiltschko, R. (2001): Computer modelling of pigeon navigation according to the “Map and Compass”-Model. Orientation and navigation – birds, humans and other animals. Paper 49, Oxford, Royal Institute of Navigation.

Nevitt, G.A. (2008): Sensory ecology on the high seas: the odor world of the procellariiform seabirds. The Journal of Experimental Biology 211, 1708-1713.

Papi, F., Fiore, L., Fiaschi, V., Benvenuti, S. (1971): The influence of olfactory nerve section on the homing capacity of carrier pigeons. Monitore zoologico italiano 5, 255-267.

Petit, C., Hossaert-McKey, M., Perret, P., Blondel, J., Lambrechts, M.M. (2002): Blue tits use selected plants and olfaction to maintain an aromatic environment for nestlings. Ecology Letters 5, 585-589.

Rieke, G.K., Wenzel, B.M. (1978): Forebrain projections of the pigeon olfactory bulb. Journal of Morphology 158 (1), 41-55.

Roper, T.J. (1999): Olfaction in birds. Advances in the Study of Behavior 28, 247-332.

Roper, T.J. (2003): Olfactory discrimination in Yellow-backed Chattering Lories L*orius garrulus flavopalliatus*: first demonstration of olfaction in Psittaciformes. Ibis 145, 689-691.

Smith, S.A., Paselk, R.A. (1986): Olfactory sensitivity in the turkey vulture (*Cathartes aura*) to three carrion-associated odorants. The Auk 103 (3), 586-592.

Stager, K.E. (1967): Avian olfaction. American Zoologist 7 (3), 415-420.

Walankiewicz, W. (2002): Breeding losses in the Collared Flycatcher *Ficedula albicollis* caused by best predators in the Bialowieza National Park (Poland). Acta Ornithologica 37 (1), 21-26.

Wallraff, H.G. (2004): Avian olfactory navigation: its empirical foundation and conceptual state. Animal behaviour 67, 189-204.

Wenzel, B.M. (1968): Olfactory Prowess of the Kiwi. Nature 220, 1133-1134.

Wiltschko, R., Wiltschko, W. (2003): Avian navigation: from historical to modern concepts. Animal behaviour 65, 257-272.

Wimberger, P.H. (1984): The use of green plant material in bird nests to avoid ectoparasites. The Auk 101, 615-618.

Effects of pyrazine on the reproductive system of a long-distance migrant, the collared flycatcher (*Ficedula albicollis*)

Abstract

In the breeding season of 2011 a set of experiments was conducted to examine the role of olfaction in three species of Passeriformes who are considered to have weak olfactory capacities. Preliminary results bring evidence that collared flycatchers (*Ficedula albicollis*) are able to perceive volatile cues in biologically relevant contexts. This study aimed to examine possible consequences of an external odor (pyrazine $C_4H_4N_2$) on physiological processes in the reproductive system of collared flycatchers. Experimental studies have shown that long-time exposure of domestic chicken to synthetic pyrazine affects egg mass and chick weight significantly. Here we present a new approach under semi-natural conditions, assigning a total of 26 nests of a wild population of collared flycatchers breeding in nest-boxes for the experiment. One group (n=13) was exposed to the odor of pyrazine in constant 24 h intervals from the onset of egg laying to completion of the nest scrape, while the other acted as a control (n=13). A potential effect of pyrazine was measured as reflected in variations in egg mass, clutch size and nestling weight between the experimental groups. Results failed to show a consistent significant influence of pyrazine on any of the measured variables though a trend in egg mass increase is detectable. We present possible assertions for the absence of an evident effect and discuss the potential role of pyrazines in the life of a long-distance migrant.

1. Introduction

Despite comprehensive research on avian biology in the last decades, little attention has been paid to the substantial role of the olfactory sense in many bird species. Based on the study of brain anatomy, birds were believed to be microsmatic or even anosmic, relying rather on vision and acoustics as major sensory modalities (Roper, 1999; Balthazart & Taziaux, 2009). In 1968 Bang and Cobb measured the relative olfactory bulb size in 108 different species of birds, presuming that an evolutionary enhancement of a part of the brain implies an upgrade in function (Bang & Cobb, 1968). Measurements showed that the olfactory bulb took up more than 30% in the brains of different tube-nosed seabirds and only 3% in a passerine. Olfactory sense seemed to be, following the conclusion size entails function, of primary importance in distinct water birds, turkey vultures and kiwis (Buitron & Nuechterlein, 1985), and is, based on little relative olfactory bulb ratios (under 9,7%), relatively unimportant in other groups like the Passeriformes (Bang & Cobb, 1968). Nowadays, scientists have repeatedly shown that birds, even those with small olfactory tissues, pertinently use odorous clues in a variety of contexts. Avian olfaction is now widely recognized and is documented (e.g. for reviews, see Roper, 1999; Balthazart & Taziaux, 2009) in the contexts of foraging in kakapos (Hagelin, 2004), kiwis (Wenzel, 1968) and new world vultures (Houston, 1986), nest building in Corsican blue tits (Mennerat, 2008; Petit *et al*., 2002) and starlings (Clarke & Mason,1985) or individual recognition in Antarctic prions (e.g. Bonadonna, 2011). The development of the olfactory sense in birds is actually believed to be on the same level with that in mammals (Mason & Clark, 2000).

Although the use of olfaction in behavioral contexts is no longer questioned, only little is known about physiological consequences of olfactory signals in birds. It has been shown that one family of odors, the pyrazines, influence reproductive processes in chicken (Barnea & Rothschild, 2002; Katz *et al*., 1999). When exposed to the external odor of 2-methoxy-3-isobutylpyrazine, chicks laid heavier eggs than the control group (Barnea & Rothschild, 2002). Katz *et al.* (1999) found a significant reduction in body weight in chicks that were exposed to 2-methoxy-3-isobutylpyrazine during incubation and rearing. Even though the mechanisms for this effect remain unknown, this could bring evidence for persistent influence of an outside olfactory stimulus on physiological processes in birds.

Pyrazine is a widely distributed heterocyclic aromatic organic compound, occurring worldwide in numerous plants and animals (Woolfson & Rothschild, 2011). Some members of the pyrazine family are odorless while others affiliate to the methoxyalkylpyrazines, the most powerful and persistent odors known (Moore & Brown, 1981; Moore *et al*., 1990). It is believed that in nature some pyrazines serve as intra- and interspecific warning signals that can either work as attractants or deterrents (Woolfson & Rothschild, 1990). As pyrazines are often associated with aposematic insects they eventually alert potential predators from toxic pray (Woolfson & Rothschild, 1990) but can also function as signal for ripe fruit for frugivorous birds (Barnea & Rothschild, 2002). Investigations bring evidence that pyrazines are involved in associative learning processes and birds are able to recognize pyrazines as alerting signals (Guilford *et al*., 2008).

Jemiolo & Novotny (1994) demonstrated that the exposure of juvenile house mice to 2,5-dimethylpyrazine significantly delayed sexual maturation in both sexes. These results, the studies of Katz *et al.* (1999) and Barnea & Rothschild (2002) support the assumption that outside odors can have putative influences on physiological processes, e.g. the reproductive system of birds.
In passerine birds the perception of volatile substances is suggested (Mennerat, 2008) but still remains poorly investigated. A parallel study conducted in spring 2011 maintains evidence that free-ranging collared flycatchers (*Ficedula albicollis*) are sensitive to volatile compounds added artificially to the nest. These migratory birds will, while foraging, eventually come into contact with natural occurring pyrazines, as they feed on both insects and infrequently on plants. Proposing collared flycatchers as suitable experimental subjects, the study aimed at examining the impact of an external odor of pyrazine on the reproductive system of these passerines under semi-natural conditions. Possible physiological effects of pyrazine treatment are expected to reflect in a variation of egg mass, clutch size or nestling weight. It is predicted that pyrazine treatment entails a mass gain in eggs and nestlings coinciding with a reduction of clutch size. Basal mechanisms for this effect will be discussed in the context of pyrazine relevance in the life of a long-distance migrant.

2. Materials and Methods

The study was carried out in the breeding season of spring 2011 in the north-eastern part of the Wienerwald in Vienna, Austria. In the rangy hills of mixed deciduous woodland nest boxes are accepted by various secondary cavity breeders, such as blue tits (*Cyanistes caeruleus*), great tits (*Parus major*) and collared flycatchers (*Ficedula albicollis*), which enables standardized testing conditions in a semi-natural environment. Data was collected in a sample of 26 nests of two local populations of collared flycatchers in the areas of Kolbeterberg and Buchberg, with an estimated distance of 1.7 km. Breeding begins in late April and continues until mid June, clutch size ranges from five to eight. From the onset of nest building the nest boxes were monitored every day to examine the progress of nest construction, the onset of egg laying, hatching date and number and constitution of the nestlings until fledging.
The sample of 26 nests in two study areas was divided randomly in two groups: one group was exposed to the odor of pyrazine (n=13), while the other acted as a control (n=13) and was treated with water only. The tested odor consisted of pyrazine ($C_4H_4N_2$; VWR International, LLC.), which is almost odorless to humans. Following the study of Barnea & Rothschild (2002), who proved a successful effect of the treatment on domestic chickens, 10 µl of pyrazine was dissolved in 99 ml of distilled water and 1ml ethanol. In nests randomly assigned to the experimental group, 10 ml of the pyrazine dilution was added directly into the nest boxes every 24 h, from the moment of finishing the nest scrape until the onset of breeding. In the control group 10 ml of a solution containing 99 ml of distilled water and 1ml ethanol but no pyrazine was applied to the nests in equal periods.
Immediately after completion of the clutch, the eggs of each nest were counted and weighed. Data

for nestling weight (±0,1 g) was collected from day 3 post-hatching. Individual age of the nestlings was estimated when chicks were first weighed. In the study period 9 clutches were lost due to predation, i.e. 3 during treatment (excluded from data) and 6 immediately post-hatching. Data for egg mass and clutch size was therefore collected on a total of 26 nests (170 eggs), data for nestling weight on a reduced sample of 20 nests (123 nestlings).
Prior to statistical analysis mean egg mass and clutch size were log-transformed to meet assumptions of normality. Mixed effect models (ANOVA) were performed with treatment and study area as fixed factors to show effects on mean egg mass and clutch size. Additionally, possible interactions between factors were tested. As nestling age on the day of weighting was not standardized but showed a linear relationship, an analysis of covariance (ANCOVA) with age and number of nestlings as covariates, study area and treatment as factors and nestling weight as depended variable was performed. All statistical analyses were carried out with SPSS (version 16.0) software.

3. Results

Results show (Table 1) that mean egg mass (F=1.241; p=0.277) or mean clutch size (F=0.001; p=0.978) did not differ between the two experimental groups in a total sample of 26 nests (=170 eggs). The pyrazine treatment did not have a significant impact on the measured variables. A direct comparison of egg mass between treatment and control group shows mean values of 1.77± 0.12 g in water treated and 1.82± 0.10 g in pyrazine treated nests. This implies a statistically not significant mean mass gain of 0.05 g (2,82%) of eggs in pyrazine treated nests. The results can be seen in Figure 1. Maximum egg weight in the control group was 2.01 g and 1.98 g in the treatment group.

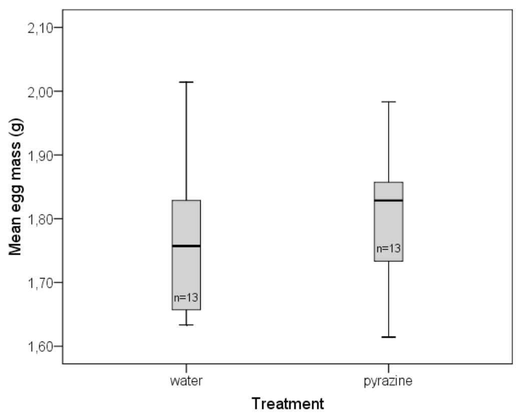

Fig. 1 Mean egg mass (g) in the control group (water) and the treatment group (pyrazine) in a total number of 26 nests.

A comparison of mean number of eggs laid by a female showed that females of both experimental groups laid an equal mean amount of eggs per clutch, 7.00 ± 0.66 in water and 7.00 ± 0.51 in pyrazine treated nests. Study area (F=0.497; p=0.488) and the interaction between study area and treatment (F=1.241; p=0.277) did not show a significant impact on mean egg mass or clutch size.
Nestling weight did not differ between control and experimental group (F=0.273; p=0.198) and is, as expected, majorly influenced by nestling age. In addition, the interaction between treatment and study area was found to be significant (F=13.226; p<0.001), indicating that the effects of pyrazine treatment on nestling weight do vary in altered environmental conditions.

Tab. 1 Effects of experimental treatment with pyrazin and other factors on egg mass, clutch size and nestling weight. d.f.: degrees of freedom.

		Egg mass		Clutch size		Nestling weight	
	d.f.	F	P	F	P	F	P
Treatment	1	1.410	0.277	0.001	0.978	0.273	0.602
Study area	1	0.497	0.488	2.039	0.167	1.673	0.198
Clutch size	1	0.105	0.901	/	/	/	/
Age of nestlings	1	/	/	/	/	198.605	<0.0001
No. of nestlings	1	/	/	/	/	0.584	0.446
Treatment*Study area	1	0.142	0.710	1.405	0.249	13.226	<0.0001

4. Discussion

Parallel to this investigation a set of experiments was conducted to examine the role of olfaction in three different species of passerine birds. Thereof one study analyzed the sensitivity to volatile compounds in collared flycatchers by documenting an alteration in behavior when adding odorous substances artificially to the nest. Immediately after the treatment, both sexes showed a significant increase in latency to enter the nest box. This adds further experimental evidence for the existence of olfactory abilities in another passerine bird and supports the notion that even birds with a small olfactory bulb are able to use olfaction. Hence collared flycatchers potentially join the line of passerine birds with assured olfactory capacities like blue tits (Petit *et al.* 2002; Mennerat *et al.*, 2004) or European starlings (Clark & Mason, 1987).
Collared flycatchers from the family of Muscicapidae are long distance migrants, breeding in southeast Europe and wintering in trans-Saharan Africa. In constantly changing environments, this insectivorous bird will eventually come into contact with pyrazines when foraging for insects, caterpillars but also berries. Based on the previous findings earlier this spring, I was encouraged to study the possible physiological effects of an outside odor, namely pyrazine, on the reproductive system of a migrating passerine. This would bring support to the findings of Barnea & Rothschild

(2002), who discovered a distinct mass gain up to 5% in eggs of chicken exposed to pyrazine odor without clearly identifying the mechanisms entailing this effect. In contrast, the experiment failed to show a significant effect of synthetic pyrazine ($C_4H_4N_2$) on any of the measured parameters. All variables (egg mass, clutch size, nestling weight) remained equal between the experimental and the control group. Nevertheless a slight trend in egg mass increase is detectable (Fig. 1), but for any further investigations and a significant conclusion greater sample sizes are needed.

The pyrazines are ubiquitous components in different plants, certain insects (mainly Coleoptera and Lepidoptera) and some terrestrial vertebrates (Woolfson & Rothschild, 1990) and are, as they often serve as attractants or deterrents, successful alerting signals for the presence of edible or inedible food (Barnea & Rothschild, 2002). With respect to the fact that pyrazines are able to improve recall of past events (Barnea *et al.*, 2004) one could presume that they play an important role in the relationship between insectivorous birds and their pray. The presence of pyrazine could therefore be a subtle volatile clue signaling the availability of potential prey for a foraging bird. This fact could be of major importance for long distance-migrants, which have to face constantly changing environments and eventually encounter pyrazines on every foraging sortie. Martin (1987) showed that supplementary feeding can affect clutch and egg size in birds, concluding that food can be seen as a limiting factor on breeding birds (Martin, 1987). The effort required to obtain the necessary resources for nestlings varies with changing environmental conditions. Parents have to determine the number and quality of offspring and 'should provide sufficient energy per offspring to optimize the chances of survival of each young to achieve the maximum number of young possible' (Martin, 1987). The availability of resources enables parents to invest more in high quality offspring, resulting eventually in a reduction of clutch size but a gain in mean egg mass. If the presence or absence of pyrazine in the environment is directly linked to the sufficient availability of potential prey it could deter or encourage parental investment resulting in a variation of egg and clutch size. To this day, such a potential mechanism is only speculation and has to be verified scientifically in all aspects.

To a certain degree egg and clutch size in birds are hormonally controlled (Scanes, 2000; Haywood, 1993). The major factors regulating both, clutch size and variation in egg mass, are the pituitary hormones FSH (Follicle-stimulating hormone) and LH (Luteinizing hormone) but also oestrogen (Johnson, 2000). Considering the results of Barnea & Rothschild (2002), no changes in oestrogen concentrations in pyrazine-exposed chicken were detected. This could obviate the influence of pyrazine on egg mass gain via hormonal interactions. In contrast, Sinervo & Licht (1991) found that FSH may be a major factor regulating clutch and egg size in female side-blotched lizards (*Uta stansburiana*). In their experiments they addressed the trade-off between egg size and clutch size and demonstrated that females treated with FSH produced larger clutches but smaller eggs than normally. Hence, for any further investigation concerning feasible physiological links between pyrazine, egg mass and clutch size, more attention should be paid to possible changes in LH or FSH levels.

However, when speculating about physiological mechanisms underlying the effect of egg or nestling mass gain, genetic preposition, ecological conditions like day length, temperature or weather, availability of food, but also age (Barnea & Rothschild, 2002), experience and constitution of the female have to be recognized as relevant extrinsic factors interacting with inner functions. Under semi-natural testing conditions standardization of environmental factors is only possible to a certain degree. This fact could be regarded as detrimental to final results, since variations in habitat quality could mask significant effects. In statistical analysis the interaction between the variables study area and treatment showed a significant impact on nestling weight. This finding gives support to the idea, that discrepancies in habitat quality, e.g. food availability, temperature, weather conditions, between the two study areas intensified or diluted possible effects of the pyrazine treatment on egg mass, clutch size or nestling weight. Since habitat quality is measurable by methods of ecology, it could easily be included in the analysis to reduce its influence on final results.

In the experiment weight of nestlings did not differ between the two groups, indicating that the exposure to pyrazine did not affect the physical constitution of the offspring. Katz *et al*. (1999) examined the effect of 2-methoxy-3-isobutylpyrazine on body weight and weight of different organs in chicken and found that long time exposure during incubation and rearing decreased body weight in both sexes, although the effect was greater in males. Contradictory are the results of Jemiolo & Novotny (1994) who showed a greater growth in male mice exposed to 2,5-dimethylpyrazine compared to non-treated mice. In the experiment females were not affected by the pyrazine treatment. In this context, the findings of Barnea & Rothschild (2002) come to mind. In their research with chicken they assessed a distinct mass gain in eggs exposed to 2-methoxy-3-isobutylpyrazine, the same kind of pyrazine used by Katz *et al.* One could assume that a rise in egg size affects chick weight positively, but no difference in body mass was found. The variations in type of physical responses to pyrazine exposure might result from different pyrazine compounds used, differences in species or variations in application periods. Since the evidence for an impact of pyrazine on body weight of nestlings in this study was lacking, it would be interesting to replace pure pyrazine ($C_4H_4N_2$) with 2-methoxy-3-isobutylpyrazine, because an effect of the latter on the body weight of birds has already been demonstrated once (Barnea & Rothschild, 2002).

In Barnea & Rothschild (2002) odor exposure of domestic chicken continued for 16 weeks. Under given conditions pyrazine exposure did not exceed 7 days limited by natural laying time of birds. Since an impact of pyrazine treatment on egg mass in chicks was measured immediately after test commencement, one could presume the same effect in collared flycatchers. Interestingly, a reduction in clutch size in pyrazine-exposed hens was only measurable in the first 4 weeks of trial.

During the study period 9 of 26 clutches were lost due to predation. Presumably accounting for a majority of losses, based on personal observations, is the Aesculapian Snake (*Elaphe longissima*) a non-venomous snake native to Austria which mainly feeds on rodents but also bird eggs as well as nestlings. In addition, other competing cavity-breeding bird species and infrequently squirrels can be seen as potential threat to clutches or young offspring. However, conspicuous is the fact that 6 of 9 destroyed nests have previously been exposed to pyrazine treatment. Theoretically the odor of pyrazine could, although the used pyrazine ($C_4H_4N_2$) was almost odorless to the human nose, somehow attract a foraging snake as they use a variety of chemosensory inputs (Shivik, 1998). It has been shown that Black Rat Snakes, a new world species of the genus Elaphe, process both visual and chemical cues to locate distant prey (Neal *et al.*, 1993), but so far the role of pyrazine in the life of wild snakes remains unknown. Further studies examining whether the olfactory presence of pyrazine can elicit appetitive behavior or stimulate approach to potential prey in Aesculapian Snakes would be useful in addressing this issue.

In summary the results of this study suggest a potential influence of pyrazine on the mean egg mass in clutches of collared flycatchers, though a significant statistical evidence is lacking. For further investigation it is essential to repeat the experiment with a larger number of birds and presumably vary the type and possible concentration of pyrazine used, since different studies demonstrate the effective application of 2-methoxy-3-isobutylpyrazine, causing a mass gain in eggs and nestlings of chicken. Finally, the basal mechanisms underlying physiological variation caused by pyrazine treatment still remain unknown. Addressing these ambiguities could be the next challenging step towards a better understanding of the role of olfactory abilities in birds.

5. References

Bang, B.G., Cobb, S. (1968): The size of the olfactory bulb in 108 species of birds. The Auk 85, 55-61.

Barnea, A., Gvaryahu, G., Rothschild, M. (2004): The effect of the odour of pyrazine and colours on recall of past events and learning in domestic chicks (*Gallus gallus domesticus*). In: Insect and bird interactions.Van Emden, H., Rothschild, M. (Eds.), Andover (UK), Intercept, 205-222.

Barnea, A., Rothschild, M. (2002): The odour of pyrazine increases the egg mass of domestic chickens (*Gallus gallus domesticus* L.). Journal of Zoology 257 (3), 411-416.

Bonadonna, F., Nevitt, G.A. (2011): Partner-Specific Odor Recognition in an Antarctic Seabird. Science 306, 835.

Buitron, D., Nuechterlein, G.L. (1985): Experiments on olfactory detection of food caches by black-billed magpies. The Condor 87, 92-95.

Clarke, L., Mason, J.R. (1985): Use of nest materials as insecticidal and anti-pathogenic agents by the European Starling. Oecologia 67 (2), 169-176.

Clarke, L., Mason, J.R. (1987): Olfactory discrimination of plant volatiles by the European starling. Animal Behaviour 35, 227-235.

Guilford, T., Nicol, C., Rotschild, M., Moore, B.P. (2008): The biological roles of pyrazines: evidence for a warning odour function. Biological Journal of the Linnean Society 31 (2), 113-128.

Hagelin, J.C. (2004): Short communication. Observations on the olfactory ability of the Kakapo *Strigops habroptilus*, the critically endangered parrot of New Zealand. IBIS The International Journal of Avian Science 146 (1), 161-164.

Haywood, S. (1993): Sensory and hormonal control of clutch size in birds. The quarterly review of biology 68 (1), 33-60.

Houston, D.C. (1986): Scavenging efficiency of turkey vultures in tropical forest. Condor 88, 318-323.

Jemiolo, B., Novotny, M. (1994): Inhibition of sexual maturation in juvenile female and male mice by a chemosignal of female origin. Physiology & Behavior 55 (3), 519-522.

Johnson, A.L. (2000): Reproduction in the female. In: Sturkie's avian physiology. 5th edition, Causey Whittow, G. (Ed.), San Diego, Academic Press, 569-596.

Katz, A., Gvaryahu, G., Robinzon, B., Snapir, N., Barnea, A. (1999): The Effect of Pyrazine Odor on Body Weight and the Weight of Various Organs in Chicks (*Gallus gallus domesticus*). Poultry Science 78, 1786-1789.

Martin, T.E. (1987): Food as a limit on breeding birds: a life-history perspective. Annual review of ecology and systematics 18, 453-487.

Mason, J.R., Clark, L. (2000): The chemical senses in birds. In: Sturkie's avian physiology. 5th edition, Causey Whittow, G. (Ed.), San Diego, Academic Press, 569-596.

Mennerat, A., Bonadonna, F., Perret, P., Lambrechts, M.M. (2004): Olfactory conditioning experiments in a food-searching passerine bird in semi-natural conditions. Behavioural Processes 70, 264-270.

Mennerat, A. (2008): Blue tits (*Cyanistes caeruleus*) respond to an experimental change in the aromatic plant odour composition of their nest. Behavioural processes 79 (3), 189-191.

Moore, B.P., Brown, W.V. (1981): Identification of warning odour components, bitter principles and antifeedant in an aposematic beetle *Metriorrynchus rhipidius* (Coleoptera: Lycidae). Insect Biochemistry 11, 493-499.

Moore, B.P., Brown, W.V., Rothschild, M. (1990): Methylalkylpyrazines in aposematic insects, their host plants and mimics. Chemoecology 1, 43-51.

Neal, J.C., Montague, W.G., James, D.A. (1993): Climbing by black rat snakes on cavity trees of Red-cockaded Woodpeckers. Wildlife Society Bulletin 21 (2), 160-165.

Petit, C., Hossaert-McKey, M., Perret, P., Blondel, J., Lambrechts, M.M. (2002): Blue tits use selected plants and olfaction to maintain an aromatic environment for nestlings. Ecology Letters 5 (4), 585-589.

Roper, T.J. (1999): Olfaction in birds. Advances in the Study of Behavior 28, 247-332.

Scanes, C.G. (2000): Introduction to endocrinology: pituitary gland. In: Sturkie's avian physiology. 5th edition, Causey Whittow, G. (Ed.), San Diego, Academic Press, 437-460.

Shivik, J.A. (1998): Brown tree snake response to visual and olfactory cues. The Journal of Wildlife Management 62 (1), 105-111.

Sinervo, B., Licht, P. (1991): Proximate constraints on the evolution of egg size, number, and total clutch mass in lizards. Science 252, 1300-1302.

Wenzel, B.M. (1968): The olfactory prowess of the kiwi. Nature 220, 1133-1134.

Woolfson, A., Rothschild, M. (1990): Speculating about pyrazines. Proceedings: Biological Sciences 242, no. 1304, 113-119.

Zusammenfassung

Der Geruchssinn, die Fähigkeit zur Wahrnehmung volatiler chemischer Stoffe, ist ein ubiquitäres System in allen Vertebraten und ermöglicht in vielfältigen biologisch und ökologisch relevanten Kontexten eine Interaktion und Kommunikation mit der Umwelt. Umso erstaunlicher scheint die Annahme, Vögel verfügten über keinen oder einen nur rudimentär ausgeprägten Geruchssinn, auch in wissenschaftlichen Kreisen bis in die späten 1960er Jahre weit verbreitet war und sich auch in den darauf folgenden Jahren nur wenige Studien mit der olfaktorischen Wahrnehmung von Vögeln beschäftigten.

Auch heute noch sind Untersuchungen in diesem Bereich spärlich, obwohl das Potential noch längst nicht erschöpft ist und Erkenntnisse zur Wahrnehmung volatiler Substanzen in vielen Bereichen der Ornithologie völlig neue Perspektiven eröffnen würde. Rezente Studien beweisen, dass die Anzahl funktionsfähiger OR Gene (olfactory-receptor-genes), also jenen Teilen der DNA die für die Geruchsrezeptoren im olfaktorischen Epithelium codieren, in den untersuchten Vogelarten wesentlich höher ist als bisher vermutet (Steiger *et al*., 2008). Bei Säugetieren gilt die Annahme, dass ein positiver Zusammenhang zwischen der Anzahl funktionsfähiger OR Gene und der Menge verschiedener Gerüche, die differenziert wahrgenommen werden können, besteht (Niimura & Nei, 2006). Folgt man dieser Hypothese, könnte der Geruchssinn bei Vögeln eine wesentlich größere Rolle spielen als angenommen (Steiger *et al*., 2008). Zugleich beweist eine Vielzahl morphologischer und neuroanatomischer Studien, dass das Geruchszentrum der Vögel alle grundlegenden Strukturen aufweist, die für eine sinnvolle Perzeption, Interpretation und Verarbeitung volatiler chemischer Signale unabdingbar sind (Roper, 1999).

Genetische, neurologische und eine steigende Anzahl verhaltensbiologischer Untersuchungen der letzten Jahre sprechen so eindeutig für die Existenz eines funktionsfähigen Geruchssinns bei verschiedenen Vogelarten (Balthazart & Taziaux, 2009). Aufgrund historischer Fehleinschätzungen, basierend auf dem Vergleich der relativen Größe des olfaktorischen Zentrums im Gehirn (Bang & Cobb, 1968), erhält die Ordnung der Sperlingsvögel (Passeriformes) nach wie vor wenig Aufmerksamkeit im Bezug auf olfaktorische Fähigkeiten. Ziel dieser Studie war es einerseits, neue Erkenntnisse zur Existenz des Geruchssinns bei zwei bisher nicht untersuchten Arten der Passeriformes, Kohlmeisen (*Parus major*) und Halsbandschnäpper (*Ficedula albicollis*) zu erhalten, andererseits einen Beitrag zur Untersuchung der möglichen ökologischen Relevanz der olfaktorischen Wahrnehmung im verhaltensökologischen Kontext bei Blaumeisen (*Cyanistes caeruleus*) und physiologischen Kontext bei Halsbandschnäppern zu leisten.

Die verhaltensökologischen Untersuchungen im Freiland zeigen deutlich, dass Kohlmeisen sowie Halsbandschnäpper ihr Verhalten nach experimenteller Änderung der olfaktorischen Umgebung im Nest adjustieren und vor dem Einflug in die Nestboxen deutlich länger zögern. Dies spricht für eine Wahrnehmung volatiler Substanzen bei beiden untersuchten Arten und unterstützt die Vermutung, dass die Größe des olfaktorischen Zentrums bei Passeriformes nicht ausschlaggebend für die Fähigkeit zur Olfaktion ist. Die Untersuchung liefert damit einen Beweis für die Existenz einer

olfaktorischen Wahrnehmung bei Kohlmeisen und Halsbandschnäppern, wobei die ökologische Relevanz des Geruchssinns bei sekundären Höhlenbrütern weiterhin rein spekulativ bleibt. Mögliche Bedeutungen liegen im Bereich der Prädatorenerkennung, Nahrungssuche, Eltern-Nestlings-Interaktionen oder der Orientierung und Navigation. Letzteres ist besonders im Hinblick auf besondere sensorische Modalitäten langstreckenziehender Arten, wie dem Halsbandschnäpper, von großem wissenschaftlichen Interesse.

Einen Beitrag zur methodischen Grundlagenforschung leisten die Ergebnisse der Untersuchung zur geruchsabhängigen Wahl von Nestmaterial bei Blaumeisen. Bisherige Studien beweisen (z.B. Lambrechts & Dos Santos, 2000), dass weibliche Korsische Blaumeisen stark aromatische Kräuter aktiv in ihr Nest einbringen. Dies setzt die Fähigkeit voraus volatile Substanzen wahrnehmen und aktiv unterscheiden zu können. Weiterhin ungewiss bleibt allerdings die biologische Relevanz dieses Verhaltens. Im Zuge dieses Experiments sollte durch die Applikation verschiedener Duftstoffe auf experimentell bereitgestelltes Material, das von Blaumeisen bevorzugt für den Nestbau verwendet wird, eine mögliche geruchliche Bevorzugung für bestimmte Duftstoffe untersucht werden. Da die Entnahmemengen in allen drei Versuchsgruppen konstant blieben, kann keine Aussage über den Einfluss bestimmter chemischer Bestandteile auf das Wahlverhalten bei Blaumeisen im Kontext der Akquisition von Nistmaterial getroffen oder weiterführende Schlüsse auf die Relevanz von Kräutern beim Nestbau gezogen werden. Allerdings liefert das Experiment einen wertvollen Beitrag zur Entwicklung neuer Feldmethoden und zeigt, dass ein experimenteller Aufbau in dieser Form funktionieren kann.

Weiterführende Beobachtungen hatten zum Ziel, mögliche Auswirkungen einer experimentellen Manipulation des Nestgeruchs auf das langfristige elterliche Investment zu untersuchen. Falls sich die Komplexität der Duftstoffzusammensetzung im Nest, wie in diversen Studien spekuliert wird (z.B. Lambrechts & Dos Santos, 2000), positiv auf die Fitness der Nachkommen auswirkt, könnte dies bei den Eltern zu einer Erhöhung des Investments führen. Ein energetischer Mehraufwand durch gesteigerte Fütterungsraten, wird durch höhere Überlebenswahrscheinlichkeit der Nestlinge ausgeglichen. Die Ergebnisse zeigen jedoch keine signifikante Änderung der Anflugraten der adulten Meisen im Versuchszeitraum, was teilweise den sich verschlechternden Witterungsbedingungen, wahrscheinlich aber auch der sehr geringen Stichprobengröße zuzuschreiben ist.

Der finale Teil dieser Arbeit untersuchte mögliche Auswirkungen eines externen Odors auf physiologische Vorgänge in weiblichen Halsbandschnäppern. Studien beweisen, dass sich die Präsenz des ubiquitären chemischen Stoffes Pyrazin maßgeblich auf das Eigewicht und die Gelegegröße von Haushühnern auswirkt, obwohl die basalen Mechanismen, die diesem Effekt unterliegen bisher unbekannt bleiben (Barnea & Rothschild, 2002). Vom Zeitpunkt der Fertigstellung der Nestmulde bis zum Brutbeginn wurde Pyrazin in regelmäßigen Abständen direkt in die Nester eingebracht. Anschließend sollten Eigewicht, Gelegegröße und Nestlingsgewicht Schlüsse über den Einfluss des externen Odors zulassen. Wie aus den Ergebnissen hervorgeht, blieben alle gemessenen Parameter unabhängig von olfaktorischen Einflüssen, obwohl ein leichter Trend zur Erhöhung des mittleren Eigewichts bei behandelten Nestern erkennbar ist. Eine

Wiederholung des Versuchs mit vergrößertem Stichprobenumfang ist unerlässlich.

Bis zum heutigen Stand der Forschung bleibt die Rolle des Geruchssinns bei Sperlingsvögeln rein spekulativ. Diese Studie leistet einen wichtigen Beitrag zur Erkenntnisgewinnung in diesem Kontext: Durch die Erbringung schlüssiger Nachweise zur Wahrnehmung von Duftstoffen bei zwei bisher nicht untersuchten Arten wird die Annahme, dass dem Geruchssinn eine weitaus größerer Bedeutung zugemessen werden sollte als bisher vermutet, bestätigt. Weiterführende Studien könnten beispielsweise die Fähigkeit zur Differenzierung verschiedener Duftstoffe untersuchen und dabei Schritt für Schritt weitere wichtige Erkenntnisse zum Verständnis der Relevanz des olfaktorischen Sinnes bei Vögeln beitragen.

Abstract

Although little is known about the biological relevance of an olfactory sense in birds, comprehensive anatomical, neurological and behavioral studies suggest that distinct species are able to perceive volatile substances by means of olfaction in a variety of biologically relevant contexts. Therefore the role of olfaction in birds might be of greater importance than originally thought.

In the breeding season of 2011 a set of experiments under semi-natural conditions was conducted to examine the role of olfaction in three passerine species, which are noted to posses the smallest olfactory bulbs in the avian kingdom. Since olfactory prowess is particularly well documented in blue tits a first experiment aimed to test new experimental approaches for in-field studies by evaluating potential odor dependent preferences in selection of nesting material in blue tits (*Cyanistes caeruleus*) and monitoring long-term effects of nest odor manipulation on parental investment. Major work focused on the existence of olfactory abilities in great tits (*Parus major*) and collared flycatchers (*Ficedula albicollis*) by manipulating nest odor composition and monitoring short-term behavioral responses. Results bring evidence that both species are receptive to volatile chemical substances added artificially to the nest, since latencies before entering the nestboxes increased significantly after odor treatment in both sexes. Further investigations on physiological consequences of an external odor (pyrazine $C_4H_4N_2$) did not reveal a significant effect on the reproductive system of collared flycatchers, as measured in variations in egg mass, clutch size and nestling weight. Results are astonishing and offer various possibilities for continuative research since there is no lack of conceivable application possibilities of olfaction in a bird's life.

Lebenslauf

MARLIES SPERANDIO

PERSÖNLICHE DATEN

geboren am 16.12.1984 in Feldkirch (AUT)

österreichische Staatsbürgerschaft

AUSBILDUNG

Universität Wien (A) Diplomstudium Biologie, Spezialisierung Zoologie Diplomarbeit im Bereich Ornithologie	2006 - 2012
Universität Wien (A) Bakkalaureatstudium Publizistik- und Kommunikationswissenschaft (Mitbelegung)	2006 - 2008
Symmedia Akademie, Bielefeld (D) Einjährige fotografische Grundausbildung	2005 - 2006
Höhere Lehranstalt für wirtschaftliche Berufe, Rankweil (A) Schwerpunkt Fremdsprachen (Englisch, Französisch, Italienisch). Abschluss mit ausgezeichnetem Erfolg	1999 - 2004

UNIVERSITÄRE PRAKTIKA

PP Schutz von Meeresschildkröten in der Türkei 5-wöchige Feldarbeit in Fethiye (Türkei) zum Schutz der bedrohten Unechten Karettschildkröte (*Caretta caretta*).	Juli-Aug. 2009
PP Tropenbiologie in Costa Rica Feldarbeit im Tieflandregenwald der Tropenstation La Gamba zum Farbsehen bei Euglossinen (Prachbienen).	Sept. 2009
PP Erfassung von Vogelpoplationen in Stadtparks Systematische Erfassung der Vogelpopulationen in Wiener Stadtparks.	Sept.-Dez. 2010
PP Lautsignale bei Tieren Analyse der Lautgebung von Weißhandgibbons (*Hylobates lar*) im Zoo Schönbrunn.	Sept.-Dez. 2010
Zoologisches Labor Praktikum Untersuchung zur Nektaraufnahmekapazität von Schmetterlingen	März-Juni 2010

BERUFSERFAHRUNG

Universität Wien Studienassistentin am Departement für Evolutionsbiologie	2011 - 2012
ZOOM Kindermuseum, Wien Vermittlerin im Ausstellungsbereich; Führung von Kindergruppen sowie Mitwirkung bei der Ausstellungsplanung und -entwicklung	2010 - 2012

SPRACHKENNTNISSE

Deutsch	Muttersprache
Englisch	flüssig (Schrift, Sprache)
Italienisch	Grundkenntnisse
Französisch	Grundkenntnisse

COMPUTERKENNTNISSE

MS Office	Word, Excel, Powerpoint – sehr gute Kenntnisse
Adobe Photoshop	gute Kenntnisse
Adobe Illustrator	Grundkenntnisse
SPSS	Grundkenntnisse
ArcGIS	Grundkenntnisse

EXTRACURRICULÄRE ERFAHRUNGEN und ERFOLGE

Teilnahme am Brutvogelmonitoring 2011 in Wien
im Auftrag der MA22 in Kooperation mit BirdLife Österreich

Auszeichnungen
Leistungsstipendium aus den Mitteln der Stiftungen und des Stipendienfonds der Universität Wien, 2010
Leistungsstipendium nach dem Studienförderungsgesetz der Universität Wien, 2010 und 2011

Auslandserfahrung
Vertiefung der sprachlichen Fähigkeiten und Sammlung von Arbeitspraxis während mehrmonatiger Aufenthalte in Neuseeland, Australien, USA, England, Costa Rica, Kanada, etc.

Wien, im Februar 2012